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ORİJİNAL ARAŞTIRMA

5% $NaHCO_3$ for Skin Antisepsis In Peripheral Intravenous Catheterization In Children: A Randomized Controlled Study

Çocuklarda Periferik İntravenöz Kateterizasyonda Cilt Antisepsisi için %5 $NaHCO_3$: Randomize Kontrollü Bir Çalışma

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ABSTRACT

Aim: In peripheral intravenous catheterization applications, procedural problems may cause local or systemic infections. The aim of this study was to determine the antimicrobial effect of 5% Sodium Bicarbonate in skin antisepsis before catheterization.

Material and Method: The study was randomized, single blind and experimental. In the study, skin antisepsis was used with 5% sodium bicarbonate before peripheral intravenous catheterization. Chlorhexidine gluconate 2% and alcohol 70% were used as a comparison group. The catheter was inserted after antisepsis was applied. One and 24 hours after catheterization, skin swabs were taken and analyzed. Local and systemic infection findings and vital signs were measured every 12 hours.

Results: 5% Sodium Bicarbonate showed antimicrobial effect in skin antisepsis. There was no evidence of systemic infection and vital signs were within normal limits. The most common bacterial subtype was *Staphylococcus hominis*, which was found in skin swabs of 8.1% of the children.

Conclusions: The 5% sodium bicarbonate may be an effective and cost-effective agent for skin antisepsis in children. It can be used for skin antisepsis before peripheral intravenous catheterization. The efficacy of different concentrations of sodium bicarbonate solutions should be investigated.

Keywords: Antiseptic, catheter-related infection, chlorhexidine gluconate, nursing care, peripheral intravenous catheterization, sodium bicarbonate.

ÖZ

Amaç: Periferik intravenöz kateterizasyon uygulamalarında, prosedürel sorunlar lokal veya sistemik enfeksiyonlara neden olabilir. Bu çalışmanın amacı kateterizasyon öncesi cilt antisepsisinde %5 Sodyum Bikarbonat'ın antimikrobiyal etkisini belirlemektir.

Gereç ve Yöntem: Çalışma randomize, tek kör ve deneysel olarak yapıldı. Çalışmada periferik intravenöz kateterizasyon öncesi %5 Sodyum Bikarbonat ile cilt antisepsisi uygulandı. Karşılaştırma grubu olarak %2 Klorheksidin Glukonat ve %70 Alkol kullanıldı. Kateter antisepsi uygulandıktan sonra yerleştirildi. Kateterizasyondan bir ve 24 saat sonra deri sürüntüleri alındı ve analiz edildi. Lokal ve sistemik enfeksiyon bulguları ve vital bulgular 12 saatte bir ölçüldü.

Bulgular: 5 Sodyum Bikarbonat cilt antisepsisinde antimikrobiyal etki gösterdi. Sistemik enfeksiyon bulgusu yoktu ve vital bulgular normal sınırlardaydı. En yaygın bakteriyel alt tip *Staphylococcus hominis* olup, çocukların %8,1'inin deri sürüntülerinde bulunmuştur.

Sonuç: %5 sodyum bikarbonat çocuklarda cilt antisepsisi için etkili ve uygun maliyetli bir ajan olabilir. Periferik intravenöz kateterizasyondan önce cilt antisepsisi için kullanılabilir. Farklı konsantrasyonlardaki sodyum bikarbonat solüsyonlarının etkinliği araştırılmalıdır.

Anahtar Kelimeler: Antiseptik, kateter ilişkili enfeksiyon, klorheksidin glukonat, hemşirelik bakımı, periferik intravenöz kateterizasyon, sodyum bikarbonat.

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INTRODUCTION

Intravenous (IV) therapy is one of the primary elements of modern health care. For pediatric patients, IV routes provided by catheters are necessary to sustain primary and secondary health. However, the risk of mortality and morbidity increases depending on the catheter used, the treatment applied, and the characteristics of the patient (1,2). As in any invasive procedure, there are complications related to the use of catheters, particularly catheter-related infection (CRI). CRIs are defined as the coexistence of findings of local and systemic infection in patients (3). As well as among patients, the increase in incidence of CRIs differs between cities and countries, and it ranks in the top three among hospital-associated infections (4). The incidence reported to be between 0.7-17% (5,6). Although there are many studies about infections related to the use of peripheral intravenous catheters (PIC) in adult patients, there are not as many studies in children about this subject (7,8). When the studies were evaluated, it was seen that various methods have been used for PIC in children (9-11). Since the risk factors for infections in pediatric patients, the characteristics of the catheter used, the drug administered, and the presence of connectors differs from adults, CRI in the pediatric age group requires a separate discussion (4)

It is undeniable that the main steps in the prevention of CRI are the management of PIC and the determination of an appropriate skin antiseptic. Although 70% Alcohol, 2% Chlorhexidine Gluconate (CHG), 1% Octenidine, and 10% povidone-iodine type antiseptics are options for skin antisepsis of children, 2% CHG is the most recommended and the most effective antiseptic recently (12,13). The trials that evaluated CHG usage in children, especially infants, reported that CHG bound keenly to skin proteins and had long-lasting antimicrobial effects on skin (48 hours). It was also seen that there was an inadequacy of studies in children and infants (12,13). Another research examined the efficacy of alcohol and bicarbonate and reported that the bicarbonate group had higher patient satisfaction despite the amount of germs on the skin remained same (14). It is known that 70% Alcohol dries the skin and causes skin lesions (1,15-17). Likewise, it has been reported that the absorption of povidone-iodine through the skin in children may cause many problems, particularly thyroid dysfunctions and skin problems (12,17). In line with this information, the need for a safe antiseptic solution for pediatric age group is emphasized (15-17).

In the world and in our country, sodium bicarbonate ($NaHCO_3$) solutions are used in various areas, such as oral hygiene and dental care, and $NaHCO_3$ as a skin antiseptic has not been associated with any unfavorable side effects (18,19). It has a bactericidal effect by neutralizing pH in the environment of bacteria (20). There is limited information in the literature regarding $NaHCO_3$ as a skin antiseptic, and this information seems to belong to the adult age group (14,19,21). No data were presented regarding the child age group. The aim of this study was to determine the efficacy of 5% $NaHCO_3$ in skin antisepsis before PIC in pediatric patients.

MATERIAL AND METHOD

Ethical aspect of the study

Ethics committee approval was obtained from the Istanbul University-Cerrahpaşa, Clinical Research Ethics Committee (10/07/2018/347) and the coordinating center of the hospital where the study conducted (01/10/2019/A-28), and institutional permission (05/08/2018) from the hospital chief physician and related units. Before the initiation of the study, the written and verbal consents of all children and their parents were obtained. The Helsinki Declaration was complied with in the study.

Aims and Design

The aim of this study was to determine the antimicrobial effects of 5% $NaHCO_3$ in PIC and to compare these effects with 2% Chlorhexidine Gluconate+70% Alcohol and 70% Alcohol. This single-blind, randomized controlled, experimental study was registered with ClinicalTrials.gov (ClinicalTrials number NCT04821193). The CONSORT flowchart was used to conduct the study (**Figure 1**).

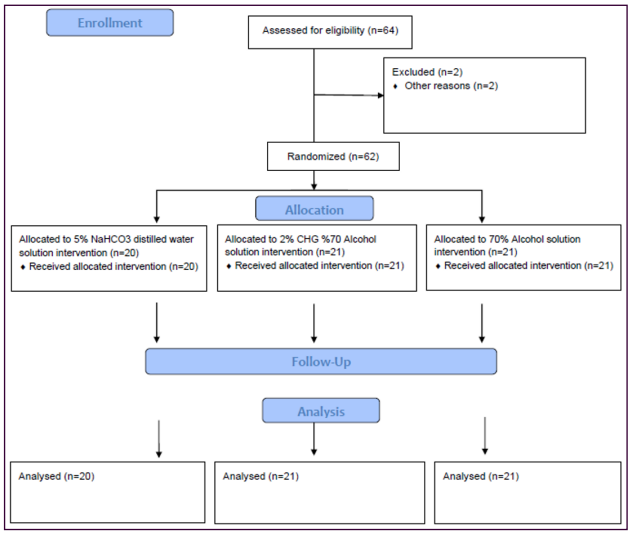

Figure 1. Study flow chart CONSORT

Participants

Children hospitalized in the pediatrics (general pediatrics and pediatric surgery) clinics of a university hospital between August 2018 and January 2019 participated in this study. The population of the study consisted of children who were in the hospital on the specified dates, and the sample size was calculated with the power analysis program. Since there was no literature for a pediatric sample, infection findings in similar studies conducted in adult patients were used to calculate the

sample (14,22). According to this calculation, a total of 57 patients were needed in the three groups, with at least 19 patients for each group (df=54; F=4.020). The groups were defined as Group 1 (% $NaHCO_3$ distilled water solution), Group 2 (2% CHG solution), and Group 3 (70% Alcohol solution). Participants were assigned to the groups through randomization using program (via Randomizer.org).

Patients in the age group of 1-18 years who were treated in inpatient clinics and required IV therapy were included in the study, while patients with a history of skin disease, immunologic disease and allergy were excluded. One patient was excluded at the second hour and one patient was excluded at the twelfth hour due to deterioration in their medical condition. Significance was accepted as $p<0.05$ at 95% confidence interval. At the end of the study, a total of 62 patients were enrolled.

Data collection tools

Data collection form and catheter infection follow-up form were used for data collection. The data collection form included questions about the children such as age, gender and medical diagnosis. It also includes catheter-related information such as the number of the catheter inserted, the site of catheter insertion and the duration of catheter stay. The catheter follow-up form consists of four sections.

The first part includes observation of the appearance of local signs of infection (pain, temperature increase, discoloration, discharge/drainage, tenderness, swelling and edema were observed) at the catheter insertion site. In the second part, systemic infection symptoms were evaluated (White blood cell count (WBC), C-reactive protein (CRP)). The data in this section were obtained from the patient's medical records. In the third section, vital signs were evaluated. In the fourth section, the results of skin swab analysis were recorded. Skin swabs taken at the first and twenty-fourth hours after catheterization were analyzed. The presence of growth and the type of bacteria grown were included.

Protocols

Protocols were designed in line with the literature, for preparation and application of the solutions (23-26). The procedures for PIC and sampling of post-catheterization skin swabs were designed in similar way (25,27).

Data Collection

The solutions used in the study were tested in the laboratory on the most common microorganisms on the skin and 5% $NaHCO_3$ distilled water was found to be effective.

Before the clinical study, skin antiseptics were prepared weekly according to antiseptic preparation protocols. After preparation, these solutions were analyzed in-vitro. Necessary data were collected from the patient file, health care provider and parents. As part of their routine care, PICs were applied to the patients after skin antisepsis with the prepared solutions. Vital signs, local (pain, temperature rise, discoloration, discharge/drainage, tenderness, swelling and edema) and systemic infection (WBC, CRP) findings were observed and recorded at the first, twelfth and twenty-fourth hours. Skin swabs were taken from the catheter insertion site at the first and twenty-fourth hours of catheterization and analyzed. All findings were recorded in the data collection and daily case forms. These procedures were continued until the required number of patients was reached.

Data analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25 package program (IBM Company, USA). After the normality distribution of the data was determined by Kolmogorov-Smirnov test, descriptive values appropriate to the distribution were given as mean, standard deviation, median and min-max. Categorical variables were given as n (%). Chi-Square, Fisher Exact Chi-Square and Kruskal Wallis tests were used for intra- and intergroup comparisons. Significance was determined as $p<0.05$.

RESULTS

The results of this study to determine the effect of 5% $NaHCO_3$ as skin antisepsis in peripheral intravenous catheterization in children are included in this section. The study was completed with 62 PICs placed in 62 pediatric patients with a mean age of 9.4 years. **Table 1** shows the descriptive characteristics of the children and **Table 2** shows the distribution and comparison of the characteristics related to catheterization according to the groups.

The results of skin swab analysis of the solutions used are presented in **Table 3**, while the results of infection findings are presented in **Table 4**. Since blood culture analysis was not routinely performed in all patients and was completed in only three patients due to the necessity of treatment, no evidence of systemic infection was observed. Bacterial growth was observed in skin swabs in all groups except group 2. As a result, 8.1% (n=5) of all patients had growth in skin swab. In group 1, no additional local infection findings other than pain were observed. There was no statistically significant difference between the groups when systemic and local infection findings were compared ($p>0.05$; **Table 4**). When vital signs were measured at the first, twelfth and twenty-fourth hours after catheter insertion in the groups, it was observed that all vital signs were within normal limits. There was no statistically significant difference between vital signs and the solutions used in all three groups ($p>0.05$).

Table 1: Distribution and comparison of descriptive features by groups (N=62)

Features	Groups			Total (n=62)	p
	Group 1 (n=20)	Group 2 (n=21)	Group 3 (n=21)		
Age (years) Mean±Sd	8.75±5.83	9.28±3.92	10.33±3.70	9.46±4.54	*0.536
	n (%)	n (%)	n (%)	n (%)	
Gender					**0.123
Girl	9 (45)	6 (28.6)	10 (47.6)	25 (40.3)	
Boy	11 (55)	15 (71.4)	11 (52.4)	37 (59.7)	
Clinics					***0.001
Child surgery	6 (30)	0 (0)	0 (0)	6 (9.7)	
General paediatrics	14 (70)	21 (100)	21 (100)	56 (90.3)	
Diagnosis					**0.660
Acute diseases	10 (50)	8 (38.1)	6 (28.6)	24 (38.7)	
Chronic diseases	8 (40)	9 (42.9)	10 (47.6)	27 (43.5)	
Infection diseases	2 (10)	4 (19)	5 (23.8)	11 (17.8)	
Length of hospital stay (days)					**0.107
1	0 (0)	1 (4.8)	3 (14.3)	4 (6.5)	
2	4 (20)	4 (19)	0 (0)	8 (12,9)	
3	3 (15)	2 (9.5)	5 (23.8)	10 (16.1)	
4 and above	13 (65)	14 (66.7)	13 (61.9)	40 (64.5)	

*Kruskal Wallis Test; **Chi Square test; ***Fisher exact Chi Square test; p>0.05, Group 1: % $NaHCO_3$ distilled water solution; Group 2: 2% CHG solution; Group 3: 70% Alcohol solution

Table 2: Distribution and comparison of characteristics related to catheterization according to groups

Features	Groups			Total (n=62)	p
	Group 1 (n=20)	Group 2 (n=21)	Group 3 (n=21)		
At which attempt the catheter was inserted Mean±Sd	1.45±1.14	1.52±1.24	1.00±0.00	1.32±0.98	0.076
Drying time of the used antiseptic Mean±Sd	[a]9.65±2.43	[b]4.80±2.11	[c]5.95±2.65	6.75±3.14	*p<0.001 +a<c<b
Length of stay of the catheter Mean±Sd	3.75±2.12	2.61±2.24	3.09±1.94	3.14±1.83	*0.214
	n (%)	n (%)	n (%)	n (%)	
Catheter number					**0.858
22 gauge	5 (25)	4 (19)	4 (19)	13 (21)	
24 gauge	15 (75)	17 (81)	17 (81)	49 (79)	
Catheter insertion site					**0.362
Hand and wrist	7 (35)	10 (47.6)	14 (66.7)	31 (50)	
Foot and ankle	0 (0)	1 (4.8)	1 (4.8)	2 (3.2)	
Arm	10 (50)	7 (33.3)	5 (23.7)	22 (35.5)	
Leg	0 (0)	1 (4.8)	0 (0)	1 (1.6)	
Joints (knee and elbow)	3 (15)	2 (9.5)	1 (4.8)	6 (9.7)	
Connector status					**0.605
Yes	19 (95)	20 (95.2)	18 (85.7)	57 (91.9)	
No	1 (5)	1 (4.8)	3 (14.3)	5 (8.1)	
Fixation status					***0.943
Yes	6 (30)	8 (38.1)	7 (33.3)	21 (33.9)	
No	14 (70)	13 (61.9)	14 (66.7)	41 (66.1)	
Used patch stick					*** p<0.001
Silk	7 (35)	1 (4.8)	17 (81)	25 (40.3)	
Transparent	13 (65)	20 (95.2)	4 (19)	37 (59.7)	
Type of drug					***0.408
Fluid	10 (50)	7 (33.3)	6 (28.6)	23 (37.1)	
Fluid and drug	9 (45)	9 (42.9)	11 (52.4)	29 (46.8)	
Drug	1 (5)	5 (23.8)	4 (19)	10 (16.1)	
Frequency of administration of the drug					***0.134
1x1	17 (85)	12 (57.1)	15 (71.5)	44 (71)	
2x1	3 (15)	4 (19)	2 (9.5)	9 (14.5)	
3x1	0 (0)	5 (23.9)	4 (19)	9 (14.5)	
Reason for catheter removal					***0.094
Infiltration/Extravasation	3 (15)	10 (47.6)	8 (38.1)	21 (33.9)	
End of treatment	17 (85)	11 (52.4)	13 (61.9)	41 (66.1)	

*Kruskal Wallis Test; **Fisher's Exact Chi Square test; ***Chi Square test; +Bonferroni test; p<0.05, Group 1: %5 $NaHCO_3$ distilled water solution; Group 2: 2% CHG solution; Group 3: 70% Alcohol solution

Table 3: Distribution of in-vivo analysis results of used solutions

Microorganisms	Groups					
	Group 1		Group 2		Group 3	
	1st hours	24th hours	1st hours	24th hours	1st hours	24th hours
*In-vivo analysis						
Citrobacter freundii and *Klebsiella pneumoniae*	-	-	-	-	✓	-
Moraxella catarrhalis and *Streptococcus pneumoniae*	-	-	-	-	✓	-
Staphylococcus auricularis and *Enterobacter Cloacae*	-	-	-	-	✓	✓
Staphylococcus hominis	✓	✓	-	-	-	✓
Staphylococcus warneri	-	-	-	-	-	✓

Group 1: %5 $NaHCO_3$ distilled water solution; Group 2: 2% CHG solution; Group 3: 70% Alcohol solution, *At the 1st and 24th hours, the isolates are different.

Table 4. Distribution of systemic and local infection findings by groups

Features	Solution groups		
	Group 1	Group 2	Group 3
Systemic signs			
Positivity in the skin swap (/patients)	2	0	3
WBC increase	2	6	2
CRP increase	10	10	11
Local signs			
Pain	-	3	4
Temperature increase	2	-	5
Colour change	2	-	3
Drain /drainage	2	-	5
Sensitivity	2	-	3
Swelling	2	-	3
Oedema	2	-	3

Group 1: %5 $NaHCO_3$ distilled water solution; Group 2: 2% CHG solution; Group 3: 70% Alcohol solution; WBC: White blood cell; CRP: C reactive protein; CRP Reference range: <5 mg/dl; WBC Reference Range: 3,6-10,2 µl

DISCUSSION

The aim of this study was to determine the antimicrobial effect of 5% Sodium Bicarbonate in skin antisepsis before catheterization. According to the findings of the study, all three solutions were found to have antiseptic effects. However, two of the solutions (Group 2 and Group 3) were already in clinical use. The 5% Sodium Bicarbonate solution used in Group 1 was applied to children for the first time, and despite being diluted with distilled water, its antiseptic effect in skin cleansing prior to peripheral intravenous catheterization was demonstrated in this study.

Skin swab analysis on catheter inserted in 62 children were performed. Local and systemic signs of infection were not observed in Group 1, and the vital signs of the children were normal. The most effective antiseptic was determined to be 2% CHG+70% Alcohol, and the effects of 5% $NaHCO_3$ and 70% Alcohol were close to each other. As the prevalence of PICs used for examination and treatment in hospitalized patients increases, the incidence of complications such as CRI also increases (14,22). Individual factors, diagnosis, the features of the catheter, and the features of the treatments applied are the risk factors in CRI seen in children (28). In the present study, there was no statistically significant difference between the groups in terms of the individual characteristics of the children, the features for catheterization, and the characteristics of the treatment applied ($p>0.05$), and it was determined that the groups formed within the scope of the study were homogeneous (**Tables 1** and **2**). When the literature was investigated, it was seen that the descriptive characteristics and catheterization features of the children in this present study were similar (3,24,28,29), and they had no effect on the occurrence of complications.

Effective skin antisepsis should be performed before PIC, and the administered antiseptic should dry as rapidly as feasible (2,5,25,27). The antiseptic penetrates under the skin from the catheter entry point if the catheters are inserted without waiting for them to dry, and causes pain in children (29). In this study, the average drying time was 9.6 seconds for Group 1. The average antiseptic drying time was less than 10 seconds, and it was even shorter in Groups 2 and 3, in which alcohol-containing solutions used. This finding can be attributed to the volatile nature of the alcohol used and the fact that Group 1 does not include alcohol.

It can be said with all these findings that 2% CHG+70% Alcohol solution is the most effective skin antiseptic, and this might be a result of its long-lasting antimicrobial activity on the skin, broad effect, and strengthened with another antiseptic agent like 70% Alcohol (15). It is reported that there is not enough evidence for its use in children under the age of two and that the hesitancy continues because it may cause allergic reactions (3,24,27). Although there is little scientific documentation about the activity of the distilled water solution with 5% $NaHCO_3$ which is recommended as an alternative antiseptic in this study, the studies have shown that it actively cleans the oil on the catheter insertion site before PIC in adult patients, that is a more effective solution compared to alcohol, provides usage satisfaction in nurses, reduces pain in patients, and that has an effect in-vitro studies (14,29,30).

Although it is known that efficacy and safety are important criteria in skin antisepsis, the skin antiseptic included in PIC management and care packages in children should

also have these properties (27,28). In the in-vivo analysis of the antiseptics used in this study, bacterial growth was detected in 8.1% of the study population and in only two patients in Group 1, where 5% $NaHCO_3$ distilled water solution was used. When the studies are examined, it is seen that CHG solution enriched with alcohol at different concentrations (0.5%, 2%) is an effective antiseptic agent compared to other antiseptics available in the market (most commonly alcohol/povidone-iodine solutions) (3,12,13). No previous study was found on the use of 5% $NaHCO_3$ distilled water solution as an antiseptic agent in children. Considering these findings, it is clear that more studies including 5% $NaHCO_3$ are needed.

When the most common microorganisms in CRI are examined, *Staphylococcus aureus* and Coagulase Positive Staphylococcus (CoPS) bacteria, especially *Staphylococcus aureus*, are frequently found in human skin, which harbors many beneficial and harmful microorganisms (3,14,31). In case of disruption of skin integrity, CoPS causes many diseases such as sepsis and soft tissue infections (30). In this study, only *Staphylococcus hominis* grew in the group using distilled water solution containing 5% $NaHCO_3$ and *Staphylococcus hominis* was the most commonly grown bacterium in all groups. *Staphylococcus hominis* is considered a potential opportunistic pathogenic microorganism present in normal human skin flora and causes infection by entering the body through weak defense barriers and entry points of invasive devices (30). To prevent microorganisms from causing infection, it is important to provide effective skin antisepsis, monitor catheter entry sites for signs of local infection, and monitor the patient for signs of systemic infection. This also requires retesting by increasing the concentration of the skin antiseptic used or adding a booster to the same concentration.

Catheter-related infection most commonly originates from the catheter insertion site, and systemic and local infection signs and symptoms guide the detection of these infections (3,11,28). A local infection passes through the layers of the skin (epidermis or dermis), and enters the internal environment, and can cause systemic infection (23). In the present study, local infection symptoms were not observed in the distilled water with 5% $NaHCO_3$ group. Symptoms such as oedema, tenderness, and swelling were observed in the groups where 2% CHG+70%Alcohol and 70% Alcohol, and the most common symptom was a color change. No signs of systemic infection (increased CRP and WBC values, positive blood culture) were found in study groups. In another study that 251 catheters were analyzed, it was reported that skin antisepsis was provided with 70% Alcohol, and no signs of CRI were found.29 In other studies, CHG solution use for skin antisepsis and routine monitoring of the catheter site for signs of local infection were recommended. It is reported that CRI could be prevented by providing optimal asepsis and antisepsis conditions in addition to observation (12,27,28). The absence of local infection signs may be an indicator for progression of infection and causing systemic infection. It is thought that the long incubation period of the microorganism is the reason the infectious microorganism is not detected in the swab sample taken from the catheter insertion site in patients with local infection signs. It may also be the effect of the patient immune system or the drugs that the patient used.

Based on the literature review and the results of the present study, 2% CHG solution seems to be an effective skin antiseptic although a safer and more effective antiseptic is needed for children. No signs of systemic and local infection were encountered in the study Group 1 with 5% $NaHCO_3$ distilled water solution, which the effectiveness tested. In light of these findings, 70% Alcohol and 2% CHG+70% Alcohol is commonly used as antiseptics in PIC. In addition, the search for alternative antiseptics for the skin continues.

Limitations

The most important limitations of this study are its single-center design. Another limitation is the absence of similar studies that can be compared and discussed with this study. The availability of powdered $NaHCO_3$ in the form of industry and the lack of 5% $NaHCO_3$ distilled water concentration is also a limitation of this study.

CONCLUSION

In this study, it was found that the groups were similar in terms of the effectiveness of the three antiseptics used in the prevention of CRI, and the most effective antiseptic was 2% CHG+70% Alcohol. The 5% $NaHCO_3$ solution, which was used for the first time in skin antisepsis in children, was found to be an effective antiseptic agent. Sodium bicarbonate may also be a cost-effective (economic) agent for skin antisepsis before PIC use in children. It is thought that the efficacy of different concentrations of sodium bicarbonate solution can be investigated in future studies. Because this study had a single-center design and consisted a small sample size, the effect of 5% $NaHCO_3$ solution should be studied in larger populations in the future.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul University Cerrahpaşa Faculty of Medicine Clinical Researches Ethics Committee (Date: 01.10.2019, Decision No: A-28).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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