Original

Antimicrobial Effect of Alcoholic Chlorhexidine on Groin Microflora in Children

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ABSTRACT

Background: Organisms found in the groin region have been implicated in surgical site infections following groin surgery and procedures in children. This study documented the groin microflora and the effectiveness of alcoholic chlorhexidine in disinfecting the groin region before surgery in children.

Method: The prospective longitudinal study included children younger than 15 years old, who were scheduled for elective clean groin surgeries. At the operating theatre, aseptically obtained swabs of the groin region were taken at three different times: before application of antisepsis, after skin preparation and after completion of wound closure. The swabs were subjected to microbiology analysis, including identification of organism and quantitative bacterial colony count. The patients were followed up for thirty days post-operation, monitoring for the development of surgical site infection (SSI)

Results: A total of 113 children were recruited. The baseline swab revealed a positive culture in 93.3% of the patients, and *Staphylococcus species* were the only flora cultured in the groin in this study, with *Staphylococcus epidermidis* accounting for 65.5%. The alcoholic chlorhexidine antiseptic demonstrated good antimicrobial activity against the skin flora with percentage bacterial count reduction rates of 98.3% and 96.0% after skin preparation and after surgery respectively. These findings are statistically significant with P-values less than 0.001 using the Paired T-test. SSI rate after the surgery was 4.4% and *Staphylococcus aureus* was the organism implicated in 80% of the cases of SSI.

Conclusion: Staphylococcus species were the flora found in the groin region of the children in this study. Alcoholic chlorhexidine, a skin preparation antiseptic, is effective in significantly reducing bacterial colony count and SSI rate in the groin.

Keywords: groin, microflora, alcoholic chlorhexidine

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INTRODUCTION

The skin is a major source of pathogens for surgical site infection (SSI) in clean surgeries; thus, skin preparation is critical in the prevention of SSI.[1,2] The architecture of the skin with abundant folds, invaginations and specialised appendages makes it a habitat for diverse microorganisms including bacteria, fungi and viruses. The majority of these organisms are commensals and thus are mostly harmless.

Groin surgeries are quite common in children and the infection rate in groin surgeries has been reported to be about 1- 4.4%.[3,4] It is important to note that the groin, considered a partly occluded region of the body (like the axilla and toe webs), is exposed to relatively higher temperature and humidity, which favours the growth of gram-negative bacilli and *Staphylococcus spp*.[5] These organisms have been largely implicated in groin wound infections.[6] There is a continuous effort in research to determine the impact of skin microflora eradication on the reduction of wound infection rate when other factors have been eliminated.[7] This is necessitated by the need to combat SSI, which remains the second most common cause of healthcare-associated infection.[8,9]

Alcoholic chlorhexidine is a composite antiseptic agent consisting of a mixture of chlorhexidine (a biguanide) in isopropyl alcohol. It has broad spectrum of antimicrobial activities against Gram-positive, Gram-negative, aerobic and anaerobic bacteria, fungi, and viruses, thus one of the ideal agents for skin disinfection.[7] It is widely used for skin preparation before surgery or invasive procedures and is one of the recommended antiseptics by the Center for Disease Control and Prevention (CDC) and the World Health Organisation (WHO) for decontaminating the surgical site.[10,11]

This study is thus aimed at describing the groin skin microflora in children and also evaluating the antimicrobial effect of alcoholic chlorhexidine on groin microflora.

METHODOLOGY

Study design and Setting

This prospective longitudinal study was conducted in the Paediatric Surgery Unit of the Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Nigeria, between the period of June 2019 and May 2020. The OOUTH is a 420-bed tertiary health institution located in Sagamu, Ogun State, Southwestern Nigeria. It is the destination of referral from the general hospitals, primary health care facilities and the private health centers in Ogun State. It also receives patients from neighbouring states due to the ease of accessing specialist care at the OOUTH. Ethics approval was obtained from the hospital's Health Research and Ethics Committee. The study was conducted in accordance with the Helsinki Declaration of 1975 ethical standards.

Study population and sampling

The study participants were patients between the ages of 1 month and 15 years, who presented at the Paediatric Surgery Outpatient Department, and were scheduled for general paediatric groin surgery. Excluded from the study were patients with open wounds on or around the groin and those for emergency surgeries. The study employed a total population sampling method, where all eligible patients within the study time frame were recruited into the study

Data collection

The parents or caregivers of these patients were informed about the study and its protocol, and informed consents were obtained. The demographic details of the patients were recorded, including age, sex and indication for surgery. The participants avoided the use of medicated or antiseptic bathing soap for at least two weeks before the scheduled surgery to ensure stabilisation of the normal skin flora. At the operating theatre, prior to the commencement of antiseptic surgical site preparation, an aseptically obtained skin swab (pre-application swab) was taken from the groin (intended site of surgery) using a moist cotton-tipped swab stick. Skin preparation was then carried out using 5% chlorhexidine in 70% isopropyl alcohol and the skin was allowed to dry for 3 minutes. A second skin swab (post-application swab) was taken from the same site as the previous one. Then, the surgical operation was carried out as planned. Upon the completion of wound closure, just before wound cleaning and dressing, a third skin swab (post-surgery swab) was taken from the skin around the incision site (same site as the previous ones). The duration of surgery was also noted.

The swab samples were transported immediately to the microbiology laboratory and processed by the Specialist Medical Microbiologist. The specimens were inoculated into MacConkey, chocolate and blood agar plates. The agar plates were incubated at 37^0C and examined after 24 and 48 hours for the growth of organisms. The number of bacterial colonies grown was counted by macroscopic count and recorded at each stage (pre-

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application, post-application and post-surgery swabs). Then, the organisms grown were identified by colonic morphology, Gram staining and biochemical tests.

The patients were followed up at the surgical outpatient clinic for 30 days post-operation in accordance with the CDC guidelines for surveillance for SSI.[12] The wounds were inspected on Days 5, 12 and 33 post-operation, looking for evidence of SSI. Whenever SSI was diagnosed, a wound swab was also obtained for microbiology analysis, and the patient was treated appropriately.

The outcome measures were the identification of organisms and enumeration of bacteria colony count at different stages of groin swab stick application for qualitative and quantitative description of groin microflora before and after application of the antiseptic solution, as well as the detection of cases of SSI during the 30-day follow-up period.

Statistical analysis

The data obtained were collated and analysed using the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp., 2019, Armonk, NY, USA). The results were presented in tabular formats. Categorical data were described with frequency and percentages, while continuous data were described with mean and standard deviation. Student T-test was used to compare means, and a p-value <0.05 was considered statistically significant.

RESULTS

A total of 113 patients were recruited into the study: 102 males and 11 females. The age range of participants spans from 2 months to 13 years, with a mean age of 3.7 ± 3.3 years. The types of groin surgeries performed included herniotomy, ligation of patent processus vaginalis and orchidopexy for undescended testis. The duration of surgery ranged from 13 minutes to 80 minutes, with an average duration of 32.7 ± 11.3 minutes. The summary of the demographic characteristics of the participants is presented in Table 1.

The analysis of the first swab before the application of antiseptic solution revealed *Staphylococcus epidermidis* as the most common flora in the groin, recorded in 74 (65.5%) of the swabs. This is followed by *Staphylococcus aureus* (11, 9.7%) while no growth was recorded in 11 (9.7%) of the swabs. Table 2 shows the profile of organisms grown at different stages of swab stick application. The second and third swab analyses show a significant reduction in positive swab culture, and *Staphylococcus aureus* predominates among the few organisms that survived the disinfection process. The pre-application swab was further analysed based on age group, and the result is presented in Table 3. All the infants had a positive culture of either *Staphylococcus epidermidis* or *Staphylococcus aureus* or both, before the application of the antiseptics. More so, *Staphylococcus aureus* was only isolated in infants and toddlers.

A quantitative analysis of the flora colony at different stages is presented in Table 4. It shows that the mean bacterial count before skin preparation is 3.2×10^6 CFU/ml. A statistically significant reduction in bacteria colony count was observed at the two swab stages after the application of the antiseptic solution in the range of 6.9×10^4 and 1.5×10^5 respectively, with P-values less than 0.001 using the Paired Sample T-test.

Follow-up surveillance for SSI revealed that five patients developed superficial incisional SSI, giving an SSI rate of 4.4%. Furthermore, *Staphylococcus aureus was* isolated in four out of the five SSI cases, while *Pseudomonas spp* was isolated in the remaining one. These four cases of staphylococcus SSIs were among those participants who had persistent *Staphylococcus aureus* growth in the post-surgery swabs. The single case of *Pseudomonas* was in an infant.

Table 1. Demographic Characteristics of Participants

Parameter		Frequency (N= 113)	Percentage (%)
Age group	< 1yr	20	17.7
	1yr – 5yrs	69	61.1
	6yrs – 10yrs	17	15.0
	11yr – 15yrs	7	6.2
Sex	Male	102	90.3
	Female	11	9.7
Type of surgery	Herniotomy	55	48.7
	Ligation of PPV	33	29.2
	Orchidopexy	25	22.1

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PPV= Patent processus vaginalis

Table 2: Profile of Groin Microflora before and after Skin Preparation

Stages	Microorganisms cultured	Frequency (N= 113)	Percentage (%)
Pre-application swab	*Staphylococcus epidermidis*	74	65.5
	Staphylococcus aureus	11	9.7
	Staphylococcus saprophyticus	8	7.1
	Staph. aureus (MRSA)	5	4.4
	Staph epidermidis + aureus	4	3.5
	No growth	11	9.7
Post-application swab	*Staphylococcus aureus*	5	4.4
	Staphylococcus epidermidis	2	1.8
	No growth	106	93.8
Post-surgery swab	*Staphylococcus aureus*	8	7.1
	Staphylococcus epidermidis	3	2.6
	No growth	102	90.3

Staph = Staphylococcus, MRSA= Methicillin-Resistant Staphylococcus Aureus

Table 3: Analysis of Pre-skin Preparation Groin Flora based on Age Group

Age group in Years – frequency (%)					
Organism	**< 1**	**1 - 5**	**6 – 10**	**11 - 15**	**Total**
Staphylococcus epidermidis	15(75)	43(62.3)	11(64.7)	5(71.4)	74(65.5)
Staphylococcus aureus	4(20)	7(10.1)	0 (0)	0 (0)	11(9.7)
Staphylococcus saprophyticus	0(0)	7(10.1)	1 (5.9)	0 (0)	8 (7.1)
Staph. aureus (MRSA)	0(0)	2(2.9)	3(17.6)	0 (0)	5 (4.4)
Staph. epidermidis + aureus	1(5)	3(4.3)	0 (0)	0 (0)	4 (3.5)
No growth	0(0)	710.1)	2(11.8)	2(28.6)	11(9.7)
Total	20(100)	69(100)	17(100)	7(100)	113(100)

Staph = Staphylococcus, MRSA= Methicillin-Resistant Staphylococcus Aureus

Table 4: Bacterial Count Reduction Pattern after Application of Antisepsis

	Mean bacteria count (CFU/ml)	**Bacteria count reduction rate (%)**	**Paired T-test**	**P-value**
Pre-application swab	3.2×10^6			
Post-application swab	6.9×10^4	98.3	13.18	<0.001
Post-surgery swab	1.5×10^5	96.0	13.09	<0.001

CFU = Colony Forming Unit

DISCUSSION

Groin flora is of particular interest to surgeons and physicians who perform surgical operations or invasive procedures in the region. Various organisms have been isolated from groin colony studies. These include Gram-positive cocci of staphylococcus species such as *Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus lugdunensis*.[13–15] These are normal flora except *Staphylococcus aureus,* which is pathogenic but often isolated in groin colony studies.[5] Other organisms that have been isolated from the groin include Gram-negative rods (*Pseudomonas, Escherichia coli), Propionibacterium, Corynebacteriu*m [5,16]. In this study, skin flora isolated from the groin were only *Staphylococcus species*, with *Staphylococcus epidermid*is being the most predominant, a finding similar to that of Powell et al.[17] However, since this research is focused on children with diversity in stages of development, microenvironments and clothing patterns –factors which are known to affect skin flora [5,18], it is worth noting that no Gram-negative

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organism was cultured, despite the fact that 18.6% of the participants in the study were infants. This is contrary to the report by Widmer et al [19] that Gram-negative enterococcal organisms are a significant component of groin microflora. Powell et al [17] also reported some Gram-negative organisms in their study, although they took swabs from both the groin and perineum, unlike in our study, where swab taking was limited to the groin region. In addition, the microbiology study in this research was limited to aerobic analysis; thus, the presence of anaerobic colonies in the groin could not be ascertained.

The finding of significant colonies of pathogenic *Staphylococcus aureus* in the groin (in up to 13.3% of subjects), as well as the fact that other coagulase-negative staphylococcus which are normal commensals may also become pathogenic when the skin barrier is breached by surgical incision, underscores the need to ensure eradication of skin flora before making an incision. The result from this study demonstrates the effectiveness of alcoholic chlorhexidine in reducing groin bacterial colony count in children. The bacteria count reduction rates in this study, as shown in Table 4, are as high as 98.3% and 96% for the post-application and post-surgery swabs. This finding is similar to that of Wong et al, [20] who reported 99% bacteria count reduction rate after the application of alcoholic chlorhexidine for arm disinfection. Meanwhile, Sistla et al [21] reported a much lower bacteria count reduction rate of 82% following skin preparation with alcoholic chlorhexidine in clean surgeries. It should be noted that both Wong et al [20] and Sistla et al [21] studied the adult population.

Analysis of the post-application and post-surgery swab culture morphology shows that *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were quite susceptible to the alcoholic chlorhexidine antiseptic, while *Staphylococcus aureus* demonstrates significant resistance to the antiseptic agent as shown in Table 1. Ramirez et al [22] reported similar findings with *Staphylococcus epidermidis* but noted that anaerobes were the organisms demonstrating significant resistance to alcoholic chlorhexidine and alcoholic iodine used in their study. Regrettably, our study did not include anaerobic analysis for comparison.

A low SSI rate of 4.4% recorded in this study is not surprising because the cases involved in the study were all clean surgeries with low expected infection rate. The SSI rate is also similar to the reports of Nakashima et al [3] and Wood et al.[4] Furthermore, surgical site infections in this study were largely due to *Staphylococcus aureus* (4/5) with a single case by *Pseudomona spp*. This finding is consistent with previous reports by Usang et al [23] and Owen et al, [24] where *Staphylococcus aureus* was recognised as the most common cause of SSI in a similar group of patients. As noted earlier, all the four cases of *Staphylococcus aureus* SSI were recorded in patients with persistent *Staphylococcus aureus* growth in their subsequent skin swabs after skin preparation. Thus, it is desired that the skin antiseptic agent should be able to completely eradicate *Staphylococcus aureus* to completely curb SSI in groin surgeries and procedures. This study thus suggests that skin flora resistance to antiseptic skin preparation may impact significantly on subsequent development of SSI, contrary to the finding of Daeschlein et al [25] who reported an unexpectedly high level of skin flora resistance to alcoholic antiseptic (up to 46%) but with no impact on subsequent SSI rate, as well as Hamzaoglu et al [26] who noted that SSIs were caused by organisms other than skin flora in their study. This difference in observation may be due to variation of the site of study; Hamzaoglu et al [26] studied the umbilicus while Daeschlein et al [25] studied many parts of the body together. The singular case of *Pseudomonas spp* SSI in this study was seen in an infant whose earlier swabs did not grow such organism, suggesting that the infection was likely due to a later contamination of the wound by the enterococcal organism from the perineum since the patient was still on diaper.[23]

Limitation: Microbiology analysis in this research work did not include anaerobic study due to the limited capability of our local laboratory for such study.

Implications of the findings: The findings of this study suggest that *Staphylococcus species* are important skin flora of note in groin surgeries and procedures. Also, while alcoholic chlorhexidine demonstrates effectiveness in eradicating groin flora, resistance to the antiseptic by *Staphylococcus aureus* still poses a significant threat.

Conclusion

Staphylococcus species are the organisms found to colonise the groin of children presenting for elective groin surgery in this study. Alcoholic chlorhexidine, as a skin preparation antiseptic, is effective in significantly reducing bacterial colony count in the groin. Staphylococcus aureus is the most common aetiology of SSI in groin wounds.

Declarations

Ethical Consideration: This study was conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1975, as

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revised in 2000. Ethics approval was obtained from the hospital's Health Research and Ethics Committee with protocol number OOUTH/HREC/212/2018AP

Authors' Contribution: Amosu, LO was responsible for research conceptualisation, clinical management, data management, manuscript writing and lead coordination. Nwokoro, CC performed research protocol review, manuscript writing and review. iii. Talabi, AO performed research protocol review and manuscript review. Lawal, IO performed laboratory protocol coordination and manuscript review. Adekoya, AO was involved in the research protocol review and manuscript review. Akpaette, IC performed clinical management and manuscript review.

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