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Microbiological Analysis of the Preputial Slit in Anambra State Nigeria: Why Neonatal Circumcision?

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Abstract

Background: Circumcision is one of the most widely performed and controversial procedures globally. While often carried out for sociocultural and religious reasons, it is also promoted as prophylaxis against urinary tract infections (UTIs), sexually transmitted infections (STIs), and HIV. The role of the preputial microbiome in these indications remains debated.
Objective: This study aimed to assess and characterize microbial species from the preputial sac of neonates undergoing circumcision and to determine their antibiotic susceptibility patterns.
Methods: A prospective cross-sectional study was conducted at a health facility between September and November 2024. Thirty-six male neonates undergoing circumcision were recruited. Swabs from the preputial sac were cultured on selective media, and isolates were identified by Gram staining and standard biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to CLSI guidelines. Data on neonatal and maternal factors were collected through structured proforma.
Results: Eight different bacteria species were isolated, with *Coagulase-Negative Staphylococcus* and *Escherichia coli* being most common (22% each). Six samples (16.7%) showed no growth. Gentamicin exhibited the highest sensitivity (50%), while high resistance rates were recorded for ampicillin and amoxicillin-clavulanic acid (80.6%). Maternal and neonatal factors, including prior antibiotic use, maternal infection, or hospital admission, did not significantly influence microbial presence or antibiotic susceptibility.
Conclusion: The preputial sac harbors primarily commensal microorganisms, with high levels of antibiotic resistance observed. Findings question the justification of neonatal circumcision as prophylaxis for UTI or STI prevention and highlight the need for reconsideration of its routine practice.

Keyword: Microbiota, preputial microbiology, prepuce, urinary tract infection, UTI, sexually transmitted infections, STIs,

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INTRODUCTION

Circumcision is the surgical removal of some or the entire prepuce from the penis.[1] It is also called prepucectomy. The prepuce is the redundant, layered fold of skin and mucus layer covering the glans penis.[2-4] Circumcision is carried out in neonates, infants, and older children. It is also carried out in adults, especially for medical indications. The indications for circumcision include sociocultural, religious, and medical reasons. The medical indications for circumcision include phimosis, paraphimosis, balanoposthitis, preputial tumour, and preputial calculus, as well as part of some surgical procedures. However, sociocultural indication is by far the highest indication for Circumcision. It constitutes over 90% of the reasons for Circumcision in children and adults. Prophylaxis against Sexually transmitted diseases (STDs) and cervical cancer has gained increased discussion as an indication for Circumcision. [2,5-8]

The preputial slit or space is thought to harbor micro-organism and increases the risk for urinary tract infection (UTI) in uncircumcised boys. It is now being projected as a prophylactic indication for circumcision. This microbiome is what is thought to increase the risk of STDs and HIV infection in uncircumcised sexually adult males.[9,10]

Circumcision is the world's most controversial surgical procedure.[1-2,7,11]. First, the controversy stems from the indications for the procedure, as socio-cultural reasons outstrip other indications.[1-2,8] The second controversy is on the execution of a life-changing procedure on a non-consenting person or before the age of consent. In most parts of Europe, circumcision for non-consenting males is frowned upon. The third controversy comes from the loss of preputial tissue. It is a specialised mucocutaneous tissue that provides coverage for the penis.

Circumcision is essentially a sociocultural practice imposed on medicine. Medicine has not been able to critically appraise this procedure and make an informed decision and advocacy.[18] This is contrary to the tenets and principles of modern medical education, which vouch for scholarship. Modern medical education insists that practice must change with the results of research. This has not been so with the practice of circumcision. Some of the "medicalized" indications for circumcision is being questioned. Phimosis can now be treated by topical medications. UTI in boys is not an epidemic. Besides proper hygiene and handling of the prepuce may influence the flora in the preputial space and thereby theoretically reduce the risk and incidence of UTI and sexually transmitted infection (STIs) in uncircumcised males.

The aim of this study therefore is to assess and characterise microbial species isolated from the preputial sac in the neonates undergoing circumcision. It will also determine the antibiotic susceptibility patterns (antibiogram) of the isolated microorganisms during neonatal circumcision and relate it to the relevance of prepuce for normal human function and, therefore, canvass an argument for or against the continual practice of neonatal circumcision as a routine procedure.

Patients and Methods

This study was designed as a prospective observational study involving male neonates undergoing circumcision. It was conducted at Immaculate Heart of Mary Specialist Hospital Nkpor Anambra State between September and November 2024. The research focused on analysing the prepuce (Foreskin) excised during circumcision. Each specimen was examined both macroscopically and microscopically to assess its structural and histological features.

Ethical Considerations

Ethical approval was obtained from the Research and Ethics Board (NAUTH/CS/66/VOL.16/VER.3/95/2024/029). Written informed consent was obtained from each neonate's caregiver before recruitment.

Eligibility Criteria

Inclusion: All male neonates undergoing circumcision by either the Plastibell or freehand technique, whose caregivers provided informed consent.

Exclusion: Neonates undergoing repeat circumcision, those with congenital penile anomalies, or those whose caregivers declined consent.

Sample Size

The minimum sample size was calculated using a web-based sample size calculator (https://www.calculator.net/sample-size-calculator.html), applying the following parameters:
Confidence interval: 95%
Margin of error: 5%

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Expected population proportion: 99%
Population size: infinite
The minimum required sample size was 16, but a total of 36 neonates were ultimately recruited to strengthen the reliability of findings.

Recruitment and Data Collection
Eligible neonates were consecutively enrolled and assigned a unique identifier (001–100). Circumcisions were carried out according to hospital protocol. During each procedure, swab stick wet with sterile normal saline is swept round inside the preputial sac/slit and sent to the microbiologist for culture, isolation and antibiogram. A structured proforma was completed for each neonate, documenting demographic and clinical details. Variables recorded included age in days, weight in kilograms, yield of micro-organism, sensitive and resistant antibiotics.

Bacterial Isolation and Identification
1. **Culture and Preparation of Culture Media**
The media used in the processing of samples include: Chocolate agar, MacConkey agar, Nutrient agar, Mueller Hinton agar, peptone water, Kligler Iron agar (Oxoid Ltd, Basingstoke, UK), Simmons' citrate agar (Titan Biotech Ltd, India). All the media used were prepared according to the manufacturer's instructions under aseptic conditions.
2. **Isolation and Morphological Identification of Isolates**
Respective non-duplicate swab samples from the patients' prepuce were cultured first on Chocolate agar and MacConkey agar. All the inoculated plates were incubated at 37°C for 18-24 hours, and growth was evaluated on these media. The identification of the isolates began with a report on **colonial morphology** on the culture media and then **Gram-staining** reactions. Depending on whether the Gram staining reaction for each isolate was positive or negative, the following biochemical tests were then conducted for further identification: **Catalase, Coagulase, Indole, Citrate Utilisation, urease test, Sugar Utilisation** (with Kligler Iron Agar-KIA) tests using standard techniques for identification of organisms. A motility test and Voges-Proskauer test were also done to determine if the organism is a Gram-negative bacillus.
3. **Antimicrobial Susceptibility Testing (Antibiogram)**
Commercially available antimicrobial discs (Oxoid Ltd, Basingstoke, UK) were used to determine the antibiotic susceptibility and resistance pattern of the isolates. The susceptibility tests were performed using the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar in accordance with the CLSI guideline (CLSI, 2022).

Two to three discrete colonies of an overnight culture of the test bacterium were touched with a sterile wire loop and suspended in about 3 mL of sterile physiologic saline (Direct colony suspension). The suspension was subsequently adjusted to match the turbidity of a 0.5 McFarland turbidity standard equivalent to 1.5×10^8 CFU/ml. The suspension was then inoculated by making a lawn on the surface of Mueller-Hinton agar plate(s) using sterile swab sticks and left to dry for 3-5 minutes. Following this, the antibiotics were placed on the medium no less than 24mm apart from each other, from the centre of one disc to the centre of another. Then the plates were incubated aerobically at 370 °C for 16-18 hours.

The 0.5 McFarland turbidity standard was prepared by adding 1 ml of concentrated tetraoxosulphate (VI) acid (H2SO4) to 99 ml of distilled water and dissolving 0.5g of dehydrated barium chloride (BaCl2.2H20) in 50 ml of distilled water in separate reaction flasks, respectively. Then 0.6 ml of the Barium chloride solution was added to 99.4 ml of the tetraoxosulphate (VI) acid solution in a separate test tube, and then the reaction mixture was mixed well. A portion of the suspension was then transferred to a capped test tube similar to the tube used for preparing the test microorganisms (Cheeseborough, 2009).

The various antibiotics to be used were selected from the 2022 recommendations of the Clinical and Laboratory Standards Institute (CLSI 2022). These include: Amipicillin (AMP:10µg), Cefoxitin(FOX:30µg), Cefuroxime (CXM:30µg), Ceftazidime (CAZ:30µg), Ceftriaxone (CRO:30µg), Cefepime (FEP:30µg), Ciprofloxacin (CIP:30µg), Gentamicin (CN:30µg); Amoxicillin - Clavulanic acid (AMC:30µg), and Trimethoprim- sulfamethoxazole (SXT:1.25/ 23.75µg).

The inhibition zone diameter (IZD) of each antibiotic produced by the isolates was then measured in millimetres (mm), and this was considered as susceptible, intermediate or resistant to the test antibiotics based on the documented breakpoint guidelines of the CLSI standard interpretive criteria (CLSI, 2022). The test was controlled using a control

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strain of *Escherichia coli* ATCC®* 25922 and *Staphylococcus aureus* ATCC®* 25923.

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RESULT

There were thirty-six (36) neonates recruited and circumcised for this study. Their prepuce was collected and analysed. The age range of the neonates was 7 to 28 days. The mean weight of the neonates was 3.6 (±0.65). These parameters are shown in Table 1.

TABLE 1: ANTHROPOMETRY OF SUBJECTS

Measures	Men (SD)	Min.	Max
Number of Subjects	36	36	36
Age in days	14.6 (±6.68)	7.0	28.0
Weight in Kg	3.6 (±0.65)	2.0	5.5

In the analysis of the possible factors that may influence the microbiome of the preputial space, it was noted that 4 (11.1%) of the neonates have used antibiotics since birth and 1 (2.8%) has been admitted into the hospital since birth. None has urethral instrumentation since birth. Thirty-three of the neonates (91.7%) were delivered at term. None has had fever since birth. Four mothers (11.1%) had maternal infection at the 3rd trimester. Three mothers (8.3%) used antibiotics in the 3rd trimester. Twenty-five mothers of the neonates (69.4%) adhered to their antenatal medications. These are shown on the Table 2.

TABLE 2: FACTORS THAT MAY AFFECT MICRO-ORGANISM IN PREPUTIAL SLIT

Factors		Frequency	Percentage
Antibiotics Use Since Birth	Yes	4	11.1%
	No	32	88.9%
Admission Since Birth	Yes	1	2.8%
	No	35	97.2%
Urethral Instrumentation	Yes	0	0%
	No	36	100%
Maternal Infection/Illness in Third Trimester	Yes	4	11.1%
	No	32	88.9%
Maternal Antibiotics Use in Third Trimester	Yes	3	8.3%
	No	33	91.7%
Gestational Age	Term	33	91.7%
	Pre-term	3	8.3%
Adherence to Antenatal Medication	Yes	25	69.4%
	No	11	30.6%
Fever Since Birth	Yes	0	0%
	No	36	100%

In the first isolates from the preputial space, eight different micro-organisms were cultured, with Coagulase Negative Staphylococcus (CONS) and *Escherichia coli* being the most, 8 (22%), respectively. Six (16.7%) of the preputial spaces showed no growth of micro-organism. These are shown in Figure 1.

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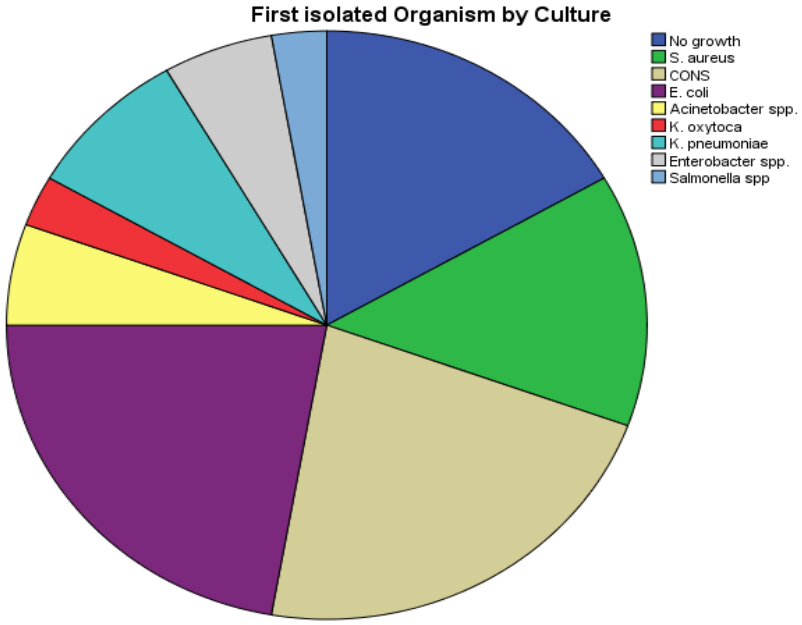

Figure 1: First Isolation of Bacteria from Preputial Space

Five (13.9%) of the prepuce showed a second isolate of bacteria. Coagulase Negative staphylococcus was the most cultured, 3 (8.3%). These are shown in Figure 2.

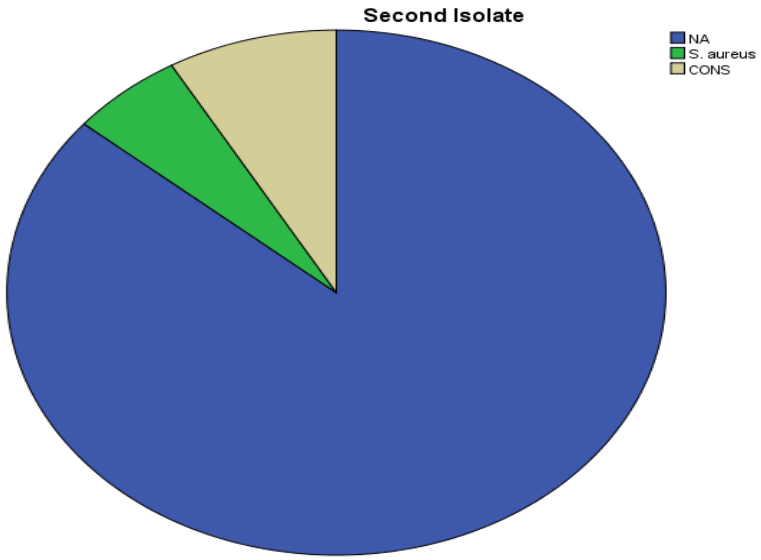

FIGURE 2: Second Bacterial Isolation from the Preputial Space

The isolates were subjected to sensitivity test for twelve antibiotics. The most sensitive antibiotics was gentamicin, to which 18 (50%) were sensitive, 9 (25%) were resistant and 9 (25%) not tested. The next most sensitive antibiotics was etapenem,

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FIGURE 2: SECOND BACTERIAL ISOLATION FROM THE PREPUTIAL SPACE

10 (27.8%). The most resisted antibiotics was seen with ampicillin and amoxicillin-clavulanic, 29 out of 30 tested (80.6%) respectively. These are shown on Table 3 below.

TABLE 3: Antibiotics Sensitivity of Isolated Micro-Organism

Antibiotics	Not Applicable	Sensitive	Resistant	Total
Cefoxitin	6 (16.7%)	8 (22.2%)	22 (61.1%)	36 (100%)
Cefuroxime	20 (55.6%)	5 (13.9%)	11 (30.6%)	36 (100%)
Ceftriaxone	20 (55.6%)	2 (5.6%)	14 (38.9%)	36 (100%)
Trimethoprim-Sulfamethoxazole	6 (16.7%)	3 (8.3%)	27 (75%)	36 (100%)
Gentamicin	9 (25%)	18 (50%)	9 (25%)	36 (100%)
Ampicillin	6 (16.7%)	1 (2.8%)	29 (80.6%)	36 (100%)
Ceftazidime	23 (63.9%)	4 (11.1%)	9 (25%)	36 (100%)
Ciprofloxacin	8 (22.2%)	4 (11.1%)	24 (66.7%)	36 (100%)
Piperacillin-Tazobactam	20 (55.6%)	12 (33.3%)	4 (11.1%)	36 (100%)
Etapenem	20 (55.6%)	10 (27.8%)	6 (16.7%)	36 (100%)
Amoxicillin-Clavulanic Acid	6 (16.7%)	1 (2.8%)	29 (80.6%)	36 (100%)
Azithromycin	19 (52.8%)	7 (19.4%)	10 (27.8%)	36 (100%)

The presence or absence of bacteria in the preputial space was not influenced or determined by prior hospital admission, antibiotics usage, and maternal use of antibiotics in the third trimester. These are shown in Table 4.

TABLE 4: Logistic Regression of Determinants of Presence of Isolate in Preputial Space

Determinants		Odd ratio (OD)	Sig	95% CI
Antibiotics use by neonate	Yes = 4 No = 32	613524897.9	0.999	0.000 - .
Hospital admission	Yes = 1 No = 35	0.376	1.000	0.000 - .
Maternal Infection	Yes = 4 No = 32	1.000	1.000	0.000 - .
Maternal Antibiotics use in 3rd trimester	Yes = 3 No = 33	0.143	1.000	0.000 - .
Gestational Age (GA)	Term = 33 Pre-term = 3	.000	0.999	0.000 - .
Adherence to antenatal visit	Yes = 25 No = 11	0.342	2.625	0.000 - .

The use of antibiotics in the neonatal period before the procedure did not influence the susceptibility of the isolated bacteria to antibiotics. The significant level (P-value) ranged from 0.999 to 1.000, at a 95% Confidence Interval (CI). The susceptibility test to the twelve (12) antibiotics was also not determined by maternal use of antibiotics in the third trimester. The significant level (P-value) ranged from 0.998 to 1.000 at 95% Confidence Interval (CI).

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DISCUSSION

Neonatal circumcision has been in practice since the antiquity. The discussion has always centered on the indications, benefits, complications and contra-indications. This study is a look into one of the touted reasons for neonatal circumcision vis-à-vis prophylaxis for urinary tract infection and sexually transmitted infections (STI).

A total of thirty-six neonates were involved in this study. They were all neonates in line with the age that seek for circumcision in the environment.[13] These were children and persons incapable of giving consent for the procedure. The procedure was performed for socio-cultural reasons. Neonatal circumcision raises complex ethical and public health questions, particularly regarding age and the lack of informed consent from the client. Circumcision in neonates is thought to reduces the risk of urinary tract infections, penile cancer, and transmission of some sexually transmitted infections (STIs), including HIV. These benefits are more pronounced when the procedure is performed early in life due to lower complication rates and greater protective effects over a lifetime, safer, and less psychological impacts.[14-15] Arguments have been advanced why circumcision should not be tolerated at the neonatal age group. Neonates cannot provide informed consent for an elective, irreversible procedure, with still a possibility of complication. It has also been proved that all the medical indications for circumcisions are preventable, if the mothers are thought how to care for the prepuce.

There are wide variety of bacteria isolated from the preputial slit in this study. The most common organisms are normal flora of the skin: staphylococcus spp. The others were probably contaminations from the anorectum. It is noted that all the neonates wear diapers which makes the genital area to be contaminated by faeces. In all, this is colonization without infection. In 6 of the neonates, no organism was isolated from the preputial space. This opens the possibility that as the child grows and manages his own hygiene, it will be possible to keep the gastro-intestinal flora away from the preputial space. This is in keeping with the findings of Zuber et al[16] in "Human Male Genital Tract Microbiota". They identified many of the organisms in the human male genital tract, from the penile coronal sulcus, urethra, prostate, testis and seminal vesicles. They also noted that these organisms can cause genito-urinary tract infection such as acute and chronic prostatitis mainly caused by *Escherichia coli*, along with other Enterobacteriaceae (*Klebsiella* spp., *Proteus* spp., and *Pseudomonas aeruginosa*), *Enterococcus* spp., and *Staphylococcus aureus*. They also isolated *Staphylococcus*, *Enterococcus*, *Escherichia,* and *Ureaplasma* gen era from the human semen in the absence of infection. These organisms were also associated with urinary tract infection and sexually transmitted infection.[16]

In the review by Tuddenham et al[17] they noted that anatomy is a major determinant of the genital microbiota in men. They discovered that the foreskin is a unique physical and biochemical environment that harbors a specific microbiota different from that of the corona sulcus. They posited that the removal of the foreskin during male circumcision causes dramatic changes in the penile microbiota. Uncircumcised men, they pointed out have high penile bacterial density and high absolute abundances of anaerobic bacteria. Hence bacteria are not a problem of uncircumcision. Mandar in his thesis pointed out that "we are born 100% human but we die 90% microbial. This, he said meant there are 10x more microbial than human cells in our body, and that each of us contains 150 times more microbial than human genes".[18] Our microorganisms are collectively known as microbiota. The genomes of these microbiota act together as a living system known as the microbiome (i.e., the collection of genes in the microbiota). It should be recognized that the human microbiome is an integral component of the human body, and, on the other hand, majority (up to 80%) of the bacterial species found in the human body are uncultured or even unculturable". Hence the micro-organisms found in the preputial slit of these neonates are not essentially abnormal but an evolution of the human body.[18] This is further re-enforce by the findings in this study in which none of the activities and exposures of the neonates and maternal bodies determined or influenced the presence of bacteria in the preputial slit.

Twelve antibiotics were tested for sensitivity to the bacteria cultured from the preputial slit. The common antibiotics used for urinary organisms were not sensitive in the most part. The level of resistance was high for Cefoxitin, Trimethoprim-Sulfmethoxazole, Ampicillin, Ciprofloxacin, and Amoxicillin-Clavulanic acid. Gentamicin and Piperacillin-Tazobactam were the antibiotics with up to 30% sensitivity for the tested bacteria.

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Generally, the level and the scope of resistance to antibiotics is very high in this study. This may be due to the abuse of antibiotics and the poor antibiotics stewardship in the environment. Mukonzo et al 2013[19] and Viswanathan 2014[20] have noted the increasing development of resistance to antibiotics in the presence of proliferation of Over-the-Counter (OTC) antibiotics usage. In addition, all the isolates were not subjected to susceptibility test with all the twelve (12) antibiotics used in this study at the same time. Again, it should be noted that this susceptibility test was done in the absence of suspected infection.

Demir et al in 2020[21] conducted a study in Tukey in which they assess the microorganisms and antibiotic profile of the subpreputial space in uncircumcised boys. They divided the prepubertal boys coming for circumcision into two: those with subpreputial smegma and those without. They had more isolates in the subpreputial spaces without smegma. The isolates gotten were similar to what was obtained in this study except for additions of organisms like *Enterococcus faecalis* and *Proteus mirabilis*, which were the most common isolates in their study. These organisms were multidrug resistant mimicking what we have in this study. They used five (5) of the antibiotics used in this study in addition to others. They noted that there was no post-operative infection after the circumcision.[21]

The Arguments

Neonatal circumcision is being promoted in the medical world as a prophylaxis for urinary tract infection, sexually transmitted infections (STIs) and HIV/AIDS transmission.[22] The medical literature has been inundated with studies proving that urinary tract infection is commoner in uncircumcised boys than in circumcised boys. It is postulated that the prepuce harbours micro-organisms that can easily find their way into the urinary tract and cause infection in the urinary tract. Again, in the sexually active boys, the preputial space will harbor sexually transmitted microorganism and make it possible for them to have contact time with male external genitalia and cause infection of the male. The preputial space provides a conducive environment for anaerobic organism which induces inflammation in the preputial mucosa enabling the STDs and HIV/AIDs causing organisms to breech the mucosal barrier easily and infect the males.[17-18] All these have led to the campaign for male circumcision in order to avert these infections and cancer.

These arguments are flawed. First, urinary tract infection is not epidemic in uncircumcised boys. In this era of improved hygiene, mothers should be able to handle the preputial space well and carefully. There has not been a concomitant attempt to excise more bacterial harbouring parts of the body like the anorectum, vagina, oral cavity, and axilla. Moreover, this study like other studies have shown that there is no unusual species of bacteria in the preputial space. It is known that bacteria in such spaces as the preputial space is part of the human microbiome.[16-17] It is not something, therefore, to victimize the prepuce for. Demir et al have shown that despite the plethora of bacteria found in the preputial space of pre-pubertal boys before circumcision, there were no incidence of surgical site infection (SSI) or complications after the procedure.[21] Moreover, the quantity and scope of bacteria is lower in uncircumcised pre-pubertal boys with smegma compared to those without smegma.[21]

Proponents of neonatal circumcision argues that it ensures a population-level STI reduction; there by potentially lowering community-wide transmission rates of HIV and HPV. Hence the child participates and sacrifices for the community good. It also by this, makes for **cost-**effectiveness by reducing long-term healthcare costs related to STIs and urinary tract infections.[23-25]

Ultimately, the ethical debate hinges on balancing immediate medical and public health benefits with the rights of the child to bodily autonomy and future choice. Performing circumcision on neonates—who cannot consent—raises ethical concerns. Critics argue that it violates bodily autonomy and the right to an intact body, with lifelong consequences made without the individual's informed choice.

CONCLUSION

The preputial sac neonates harbours the usual bacteria found on the skin and perineal area, and in some none. These bacteria exhibit high resistance to tested antibiotics. These are probably from normal colonization from the environment and contamination from contiguous areas. We recommend a review of the prophylactic indication of neonatal circumcision as a means to reduce UTI and STIs. The evidence for this practice and campaign is not strong.

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