Microorganisms and Antibiotic Profile of the Subpreputial Space in Uncircumcised Boys

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Purpose: This study investigates the frequency of isolated microorganisms and the antimicrobial resistant pattern of inner foreskin and smegma in prepubertal children.

Materials and Methods: This comparative cross-sectional study was conducted between March and November 2019, where 132 prepubertal boys, who were scheduled to receive religious circumcisions at our outpatient clinic, were examined. The patients were divided into the following groups based on the presence of smegma in their subpreputial space: Group I (with smegma, n=58) and Group II (without smegma, n=74). Sterile stuart transport swabs (Advanced Diagnostic Research, Mediko Kimya, Turkey) were taken from the smegma or the subpreputial space (glans surface and inner foreskin) using aseptic techniques and then the swab samples were immediately transported by sterile stuart transportation for microscopy, culture identification, and antibiographic resistance testing by conventional test methods and automated systems (VITEK II, Biomerieux, France) to the Microbiological Laboratory of our hospital.

Results: 48 bacteria isolated from 39 boys in Group I comprised 28 gram-positive species (58.3%) and 20 gram-negative species (41.7%). The most commonly isolated gram-negative bacterium was Proteus mirabilis (45%) while most positive was Staphylococcus hominis (42.9%). In Group II, 68 boys had 103 bacterial isolates in the glans comprising 81 gram-positive species (78.6%) and 22 gram-negative species (21.4%). The most commonly isolated gram-negative bacterium was Proteus mirabilis (42.9%) while the most positive were Enterococcus faecalis (40.7%) and S. hominis (42.9%)

Conclusion: The subpreputial space of uncircumcised boys is colonized by various types of uropathogens resistant to multidrug drugs. Smegma does not pose additional risks to microbiological colonization in children.

Keywords: child; circumcision; foreskin; microbiology; smegma

INTRODUCTION

Circumcision—the routine removal of the foreskin—is the world's oldest and most controversial surgery[1] and has been done for nearly half a century[2,3]. In 1971, the American Academy of Pediatrics (AAP) opposed routine circumcision stating that it is not a valid medical procedure[4]. It's importance was reaffirmed in 1975 and later approved by the American College of Gynecology and Obstetrics[4]. Nevertheless, subsequent studies have shown that of the 0.78% of infants diagnosed with urinary tract infections (UTIs) in their first year of life, 95% are uncircumcised[5]. Furthermore, only 0.47% of female babies and 0.21% of circumcised male babies in contrast to the 4.12% of uncircumcised male babies develop UTIs[5]. Thus, uncircumcised babies are 20 times more likely to get UTIs in their first year than circumcised babies[4].

The common causative organisms of UTIs in children stem colonisation and ascending infection from intestinal flora. Additionally, in uncircumcised boys, the preputial space is also a potential reservoir for microbial agents as uropathogenic bacteria can easily colonize unretractable foreskin, which is important in the pathogenesis of UTIs[4-7]. Further, periurethral colonization is another important factor in the development of UTIs[8]. Escherichia Coli is the most common pathogen in UTIs amongst boys and girls[9]. It is worth noting that although *E. Coli* with Fimbria (most common Type 1 and P-fimbriae) can be attached to the inner mucosal surface of the foreskin, it does not adhere to the outer surface of the foreskin[10].

Meanwhile, smegma is the accumulation of desquamated epithelial cells, collected between the glans penis and the foreskin. It is cheese-like fat with a mixture of prostate gland and seminal vesicles secretion and mucin released from the urethral glands. Smegma moistens and lubricates the cavity between the glans and the prepuce, which is known as the subpreputial space[11–13]. However, several studies have shown organisms can colonize the subpreputial space cavity[13-15]. However, whether smegma is a risk factor for UTIs is still an unanswered

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Table 1. The characteristics of patients, and the type and number of uropathogens isolated from the groups.

Variables[a]	Group I (N = 58)	Group II (N = 74)	*P*-value
Age (month)	35.0 ± 22.0	33.0 ± 23.5	.856
The number of patients with bacterial isolation (n)	39 (39/58, 67.2%)	68 (68/74, 91.9%)	< 0.001
The number of patients with single bacterial isolation (n)	30 (30/39, 76.9%)	35 (35/68, 51.5%)	.009
The number of patients with mixed bacterial isolation (n)	9 (9/39, 23.1%)	33 (33/68, 48.5%)	.001
The number of patients with no bacterial isolation (n)	19 (19/58, 32.8%)	6 (6/74, 8.1%)	< 0.001
Total number of bacteria isolated (n)	48 (48/151, 31.8%)	103 (103/151, 68.2%)	<0.001
Gram (+) bacteria number (n)	28 (58.3%)	81 (78.6%)	.016
Gram (-) bacteria number (n)	20 (41.7%)	22 (21.4%)	.016
The most frequently isolated gram (+) bacterium	Staphylococcus hominis (12/28, 42.9%)	Enterococcus faecalis (34/81, 42%)	
The most frequently isolated gram (-) bacterium	Proteus mirabilis (9/20, 45%)	Proteus mirabilis (9/22, 40.9%)	

Group I, with smegma; Group II, without smegma (glans swap)
a: Fisher's exact test and Chi-Square test ($P < 0.05$)

question, but knowledge regarding local antimicrobial resistance is essential in terms of guiding empirical antibiotic usage in the treatment of UTI in children.
This article is to examine the frequency of isolated microorganisms and the antimicrobial resistance patterns of subpreputial flora through studying the smegma samples and swabs taken from the closed subpreputial space of asymptomatic, uncircumcised boys.

MATERIALS AND METHODS

Study Population

This comparative cross-sectional study was conducted at our hospital between March and November 2019 and approved by the ethics committee of the Ministry of Health, University of Health Sciences, Van Education and Research Hospital Van, Turkey (approval number: 2018–9). All patients involved in this study offered written informed consent. The 132 boys enrolled in the study were split into two groups: Group I (with smegma) and Group II (glans swabs without smegma) based on the presence of smegma in their closed subpreputial space between the inner prepuce skin and the glans surface.

Inclusion and exclusion criteria

To avoid confounding the results, we excluded patients with UTIs, including cases of balanoposthitis, phimosis, and past UTI histories14, and those who had recently taken antimicrobial or immunosuppressive drugs.

Procedures

After surgical draping in the operating room, the prepuce was aseptically retracted to expose the glans. With the patients in Group I, the smegma was obtained within the exposed subpreputial space. With the patients in Group II, who did not have smegma, the subpreputial space was swabbed.
In Group I, the smegma was removed by means of sterile surgical forceps and mixed in normal saline during circumcision. This mixture was kept in a sterile tube for smegma culture. In Group II, a sterile stuart transferring swab was taken from the mucosal surface of the foreskin and the glans within the subpreputial space.

Evaluations

The swabs were then directly sent to our hospital's

Table 2. Type and number of uropathogens isolated from groups.

Variables[a]	Group I (N = 58)	Group II (N = 74)	*P*-value
Total number of bacteria isolated (n)	48 (31.8%)	103 (68.2%)	<0.001
Gram (+) bacteria (n)	28 (58.3%)	81 (78.6%)	.016
Enterococcus faecalis	10 (35.7%)	34 (42.0%)	.720
Staphylococcus hominis	12 (42.9%)	27 (33.3%)	.498
Staphylococcus haemolyticus	0 (0%)	6 (7.4%)	.335
Staphylococcus epidermidis	1 (3.6%)	5 (6.2%)	.515
Staphylococcus warneri	2 (7.1%)	2 (2.5%)	.272
Staphylococcus aureus	2 (7.1%)	2 (2.5%)	.272
Streptococcus pneumoniae	0 (0%)	1 (1.2%)	.743
Granulicatella adiacens	0 (0%)	1 (1.2%)	.743
Micrococcus spp	1 (3.6%)	2 (2.5%)	.594
Kocuria rosea	0 (0%)	1 (1.2%)	.743
Gram (-) bacteria (n)	20 (41.7%)	22 (21.4%)	.016
Proteus mirabilis	9 (45%)	9 (40.9%)	.789
Pseudomonas florescens	0 (0%)	1 (4.5%)	.524
Escherichia coli	5 (25%)	6 (27 %)	.864
Enterobacter aerogenes	1 (5%)	2 (9%)	.537
Morganella morgani	1 (5%)	2 (9%)	.537
Klebsiella oxytoca	0 (0%)	1 (4.5%)	.524
Klebsiella pneumoniae	2 (10%)	0 (0%)	.221
Proteus hauseri	0 (0%)	1 (4.5%)	.524
Providencia rettgeri	1 (5%)	0 (0%)	.476
Citrobacter farmeri	1 (5%)	0 (0%)	.476

Group I, with smegma; Group II, without smegma (glans swap)
a: Fisher's exact test and Chi-Square test ($P < 0.05$)

Table 3. Gram (+) bacterial isolates and result of resistance test to the antimicrobial agents (%)by conventional test methods and automated systems (Bacteria number and acronym for antibiotic names)

Bacteria number (n)	TEC	AMP	CİP	LZD	DAP	VA	TGC	TMP/SXT	E	DA	DAP	TE	FOS	FA	FOX	GN	F	LEV	RİF	MOX	P
E.faecalis (44)	7.1	0	2.3	0	7.1	7.1	0	100													
S.hominis (39)	0		0	0			0	0	86.2	13.7	0	0	72.4	75.8	6.8	0					
S.haemolyticus (6)	100			100		100	0	0	100	100		100	100	100	100	100	0				
S.epidermidis (6)			0			100		0	40	100		0						0			
S. warneri (4)	0		0		0	0	0	0	100	0		100	100	0		0					
S aureus (4)		25	25					0									25	0	25		
S.pneumoniae (1)	0	100		0		0	0	0	0	0		0					0	100	0	100	0
G. adiacens(1)	0		100	0		0	100	100	100	100	100	100				100					

Notes: Among gram-positive bacteria, Kocuria rosea and Micrococcus spp: Since they are considered flora component, antibiotic sensitivity testing is not performed (see table 2)

Abbreviations: TEC: Teicoplanin, AM: Ampicillin, CİP: Ciprofloxasin, LZD: Linezolid, DAP:Daptomycin, VA:Vancomycin, TGC:Tigecycline, TMP/SXT:Trimethoprim/ sulfamethoxazole, E:Erithromycine, DA:Clindamycin, DAP: Daptomycine TE: Tetracycline, FOS: Fosfomycin, FA:Phucydic Acid, FOX:Cefoxitin, GN: Gentamycin, F:Nitrofurantoin, LEV:Levofloxasin, RİF: Riphampin, MOX-:Moksifloxasin, P:Penisilin.

microbiology laboratory for microscopy, culture identification, and antimicrobial sensitivity testing. First, the swabs were inoculated aseptically onto blood agar, chocolate agar, and EMB agar using a sterile plastic wire loop. All incubations were kept at 37 °C for 24 hours for the aerobic culture. Next, the bacteria were isolated, identified, and confirmed by standard bacteriological techniques and antimicrobial sensitivity tests (AST) using the Vitec II system (BioMérieux, Inc., Durham, NC) by EUCAST MIC Breakpoints. It was not prepared a direct smear to gram stain.

After the surgery, the patients underwent a routine follow-up scheduled for six months later.

Statistical Analysis

Statistical analysis was performed using IBM SPSS statistics ver. 22.0 (IBM Co., Armonk, NY, USA) with a Fisher's exact test and a Chi-Square test. A *P*-value < 0.05 was considered statistically significant.

RESULTS

The 132 children ranged from six months to 7 years 4 months in age (mean age: 34 ± 22.7 months). They were divided into two groups: the age of the children in Group I ranged from 1 years to 6 years 11 months (mean age: 35.0 ± 22.0 months) while the age of the children in Group II ranged from six months to 7 years 4 months (mean age: 33.0 ± 23.5 months) (*P* =.856).

Smegma samples were obtained from 58 patients (Group I). In 39 of them, 48 organisms were isolated; 30 samples had a single organism isolated (76.9%), 9 had mixed growths isolated (23.1%), and 19 had no organisms isolated (32.8%). Further, 28 gram-positive (58.3%) and 20 gram-negative (41.7%) bacteria found. The most commonly isolated gram-negative bacterium was Proteus mirabilis (9/20, 45%), while the most positive was Staphylococcus hominis (12/28, 42.9%) (**Tables1 and 2**).

Subpreputial space swabs were taken from 74 patients (Group II) out of which, 35 (51.5%) had a single organism isolated, 33 had mixed growths isolated (48.5%), and six had no bacteria isolated (8.1%); 103 bacterial uropathogens were isolated from 68 boys. These uropathogens were made up of 22 gram-negative isolates (21.4%) and 81 gram-positive isolates (78.6%). The most commonly isolated gram-negative uropathogen was Proteus mirabilis (9/22, 40.9%) while the most positive was Enterococcus faecalis (34/81, 42%) (**Table 1 and Table 2**).

Meanwhile, among the total isolates obtained from Group I and Group II, the most commonly isolated gram-negative bacterium was Proteus mirabilis (18/42, 42.9%) while the most positive were Enterococcus faecalis (44/109, 40.7%) and S. hominis (39/109, 42.9%) (**Table 2**).

However, most of the bacterial isolates were multi-drug resistant (61.8%) testing by conventional test methods and automated systems (VITEK II, Biomerieux, France) (**Table 3 and Table 4**).

It is important to note that none of the patients have any post-operative complications, such as surgical site infections (SSI) or UTIs.

DISCUSSION

A variety of organisms can colonize the subpreputial space and its smegma[13-15]. In some cases, this colonization can be the initial step in the development of a UTI[8]. Moreover, studies have shown that uncircumcised infants have a higher rate of urinary tract infections in the first few months of life as compared to circumcised infants. In this context, Ginsburg and McCracken first noted that 95% of male infants with UTIs were uncircumcised[5]. Later, in extensive retrospective cohort studies of U.S. Army dependents, Wiswell et al. documented that uncircumcised children have 10 to 20 times greater risk of UTIs in the first few months of life as compared to circumcised children[5]. Thus, it can be seen that non-circumcision is a highly significant risk factor in the development of UTIs in infants up to 12 months of age and affects infants regardless of race and socioeconomic status[9].

Further, the risk of UTIs appears to be particularly rel-

Table 4. Gram (-) bacterial isolates and result of resistance test to the antimicrobial agents (%) by conventional test methods and automated systems (Bacteria number and acronym for antibiotic names)

Bacteria number (n)	CXM	FOX	AM	CAZ	CRO	FEP	ETP	MEM	AK	GN	CİP	TGC	CO	TMP/SXT	F	FOS	PIP	TPZ	AZT	NE	TOB	LEV	IPM	CF	AX
P mirabilis (18)	0	0		0	0	0	0	0	0	0	28.5	100	100	71.4	100	0									
Pflurescens (1)				0		0		0	0	0						0	0	0	0	0	0	0			
Ecoli (11)	0	0		14.2	0	0	0	0	0	0	0	0	0	14.2	0	0	14.2							14.2	
E aerogenes (3)	100	100	100	100	100	0	0	0	0	0	0	100	0	0			100							100	100
M morgani (3)				0	0	0	0	0	0	0		100	100	0		0	0	0	0	0	0	0	33.3		
K oxytoca (1)	0	0		0	0	0	0	0	0	0	0	100	100	0				0						100	0
K pneumoniae (2)	100	0		100	100	100	0	0	0	0	0	0	0	0			0							100	100
P hauseri (1)	100	100	100	0	100	0	0	0	0	0	0	100	100	0			0							100	100
Providencia rettgeri (1)	100	0	100	100	100	100	0	0	0	0	0	100	100	0											
Citrobacter farmeri (1)	100	0	100	0	100	0	0	0	0	0	0	0	0	0			0							100	0

Abbreviations: CXM: Cefuroxime, FOX:Cefoksitin, AM: Ampicillin, CAZ: Ceftazidime, CRO: Ceftriaxone, FEP: Cefepime, ETP:Ertapenem, MEM: Meropenem, AK: Amikasin, GN: Gentamycin, C□P: Ciprofloxasin, TGC:Tigecycline, CO: Colistin, TMP/SXT:Trimethoprim/ sulfamethoxazole, F:Nitrofurantoin, FOS: Fosfomycin, PIP: Piperasilin, TPZ: Piperasilin/ Tazobactam, AZT:Azetroenam, NE: Netilmisin, TOB: Tobramisin, LEV:Levofloxasin, IPM: Imipenem, CF: Cefazolin, AX: Amoxicillin.

evant during the first six months of a child's life when there is an increased amount of uropathogenic bacteria colonizing the prepuce. In other words, the periurethral colonization of uncircumcised children seems to be an important first step for ascending UTI seems to decrease and resolve itself by the time the child is around the age of five[8]. Thus, in general, circumcision has many health benefits, including a decreased risk of UTIs as it reduces the rate of UTI development in the first six months of a child's life almost tenfold[16,17].

On the other hand, the build-up of necrotic debris under the prepuce is a common occurrence in uncircumcised children, unless the prepuce is regularly retracted and the area cleaned. This debris is popularly known as "smegma"—a word of Greek origin that means "soap" or "salve". At first, smegma was thought to be produced by ectopic subpreputial sebaceous glands near the frenulum, called the Tyson's glands, which were never found[13]. However, in actuality, smegma is a subpreputial collection of desquamated epithelial debris mixed with mucin and secretions. It has a composition that includes fat (about 27%) and protein (about 13%) and largely functions to moisten and lubricate the subpreputial space[12,13]. It also contains cathepsin B, lysozymes, chymotrypsin, neutrophil elastase, and cytokines, which may play an important role in the immune mechanism[11,18]. Fleiss et al. supported this idea by suggesting that the oligosaccharides in breast milk are excreted when a child urinates, thereby preventing E. coli from adhering to the urinary tract and inner lining of the prepuce[11]. Further, lysozyme, which originates from the prostate and seminal vesicles, destroys bacterial cell walls and inhibits or destroys candida species[19].

Despite these findings, the role of smegma in pediatric UTIs has not yet been completely understood. In a study from Nigeria[19], bacterial isolates were found in smegma swabs from 52 boys ranging from 7 days to 11 years in age; 34 boys had single bacteria isolated (65.4%), 8 had mixed growths isolated (15.4%), and 10 had no isolated bacteria (19.2%). The most commonly isolated gram-positive bacterium was Staphylococcus epidermidis (44.8%) and S. aureus (41.4%), while the most negative was *E. coli* (90.5%). The study suggested that the differences found in the organisms relative to other studies may be ascribed to local variations and socio-economic differences due to variations in climate and diet. Similar to this study's findings, most of the bacterial isolates were found to be multi-drug resistant by conventional test methods and automated systems.

In a study from Turkey, smegma swabs were taken from 100 prepubertal, healthy boys ranging from two months to nine years(20). The 72 isolates consisted of 54 gram-positive bacteria (75.0%), 17 gram-negative bacteria (23.6%), and one Candida isolate (1.4%). The most commonly isolated gram-negative bacterium was *E. coli* (41.2%), while most positive was Enterococcus sp. (57.4%). However, most of the bacterial isolates were found to be drug-sensitive. Meanwhile, when treating UTIs, higher antibiotic resistance rates were frequently determined with regards to ampicillin, nitrofurantoin, and gentamycin.

In a study from Korea, patients were classified into two groups: Group S (with smegma, n=20) and GroupC (without smegma, n=20)[5]. In Group S, 12 boys had 22 bacterial isolates in the glans. The commonly isolated bacteria were E. coli (27.3%), E. avium (22.7%), and E. faecalis (18.2%). In Group C, 13 boys had 21 bacterial isolates in the glans. The most commonly isolated bacterial uropathogens were E. faecalis (6/21, 28.6%), E.avium (2/21, 9.5%), and E.raffinosus (2/21, 9.5%). However, E. coli was isolated in just one patient from Group C. Most of the organisms isolated were sensitive to common antimicrobial agents in clinical practices, except ampicillin for gram-negative isolates and eryth-

romycin, penicillin-G, and tetracycline for gram-positive isolates. Moreover, over half (61.3%) of the organisms isolated were multi-drug resistant.
To the best of our knowledge, this study surveyed the largest number of patients when comparing smegma and glans swab culture. The number of patients with bacterial isolation, total bacteria, gram (+) and gram (-) bacteria isolated in group II were significantly higher than group I, respectively. (*P* = .001, *P* = .001, *P* = .016, *P* = .016). We demonstrated that smegma does not pose additional risk in microbiological colonization. Among all the isolates obtained from groups I and II, the most commonly isolated gram-negative bacterium was Proteus mirabilis (18/42, 42.9%), while positive was Enterococcus faecalis (44/109, 40.7%) and S. hominis (39/109, 42.9%) (**Table2**). Most of the organisms isolated were sensitive to commonly used antimicrobial agents, except ampicillin, cefazolin, and amoxicillin in gram-negative isolates, and erythromycin and fosfomycin in gram-positive isolates. Further, most of the bacterial isolates were multi-drug resistant (61.8%) (**Table 3 and Table 4**). In a study by Chung et al., 20% of patients had no microorganisms[5]. In our study, 18.9% (25/132) of our patients did not have microorganisms and were found to be compatible with the literature (**Table 1**).
Antibiotic resistance differs according to geographic locations and is directly proportional to the use and misuse of antibiotics. Understanding the effect of drug resistance is crucial because of its deep effect on the treatment of infections. Recently, these multi-drug resistant organisms have become a serious threat to regions around the world, including Turkey, and require treatment using reserve drugs. In this context, the high detection rates of multi-drug resistance in smegma and glans swabs is an interesting dimension to this study. The variety of organisms in the smegma of boys, which are multi-drug resistant, may be linked to an increased virulence in these organisms[21,22]. Thus, it is imperative that these organisms be examined and characterized before any surgical reconstruction involving the prepuce, such as hypospadias repair, as it may contribute to poor wound outcomes.
Although the diagnosis of UTIs in young children requires a semiquantitative culture of urine to be obtained by suprapubic aspiration or urethral catheterization[22,23], a subpreputial swab in uncircumcised boys may aid in the diagnostical process, given that periurethral colonization is an important prelude to ascending infections through the urethra[8].
Despite the discovery of a variety of organisms in the subpreputial space of boys, none of the patients studied were detected with UTI symptoms or postoperative UTI complications. This supports the fact that colonization does not always lead to infection.

CONCLUSIONS

The preputial space of the children we examined were colonized by various multi-drug resistant organisms including gram positive and gram negative organisms by standard bacteriological techniques and antimicrobial sensitivity tests (AST) using the Vitec II system (BioMérieux, Inc., Durham, NC) by EUCAST MIC breakpoints. The researchers believe that because the microbiology of smegma is similar to that of the preputial space, it did not present any additional risk to microbiological colonization in the children in this study.

CONFLICT OF ONTEREST

All of the authors declare that there are no conflicts of interest.

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