

Original Article

Is Aerobic Preputial Flora Age Dependent?

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(Received April 4, 2005. Accepted July 1, 2005)

SUMMARY: Urinary tract infection (UTI) is one of the most commonly encountered infections in childhood. It has been demonstrated that the preputial sac can act as a reservoir of organisms and is thus responsible for causing ascending UTIs. This study was performed to determine the presence of preputial flora in different age groups. Prepuce and urine samples were taken simultaneously from 92 uncircumcised and healthy male children aged between 0-12 years. The data were analyzed by age, with 47 subjects of 6 years of age or less, and 45 aged 7-12 years. Twenty-seven percent of the older patients had negative preputial cultures versus 8% of those under 6 years of age ($\chi^2 = 5.27$, $P = 0.02$). In addition, enteric bacteria were the most common pathogens isolated from the prepuce in younger children while skin flora bacteria were most common in the older group ($\chi^2 = 9.18$, $P = 0.002$). The urine was sterile in all cases. Preputial cultures change with age in uncircumcised boys. This change may be related to the development of immune status, to histological or anatomical changes in the prepuce, and/or to improved personal hygiene.

INTRODUCTION

Urinary tract infection (UTI) is a common bacterial infection in childhood. The pathophysiology of UTI reflects a complex interaction between virulence factors of the microorganisms and the host defence (1). UTIs in children are usually ascending rather than blood-borne (2,3) and are caused by bacteria in the gastrointestinal tract that have colonized the periurethral area. The source of UTI is usually the periurethral region (4). After birth, the periurethral area, including the distal urethra, becomes colonized with aerobic and anaerobic microorganisms (5) that appear to function as defence barriers against colonization by uropathogens (6).

The male prepuce is in an immature stage of development at birth. Separation of the prepuce from the glans begins at 24 weeks of gestation. However, the stage of preputial separation reached at birth varies greatly among individuals (7,8). Incomplete separation of the prepuce is common and normal in neonates and infants because the narrow tip of the foreskin will not pass over the glans penis, and preputial separation may not be completed until adolescence (9).

The prepuce contains muscle fibers that work to keep the tip closed when the child is not urinating. According to a previous study, the volume of smooth muscle fibers in the preputium is statistically significantly greater in the younger age group than elastic fibers in the older age group. The normal tone of the muscle arranged in this fashion and supplemented by elastic tissue offers a legitimate explanation for the close fit of the prepuce with age (10).

Epithelial Langerhans cells (ELCs), a component of the immune system, help the body recognize and process antigens by directing them to lymphocytes or macrophages, and they can secrete cytokines (11). Weiss et al. were unable to document Langerhans cells in the mucosal surface of new-

born foreskins (12), but in the adult prepuce, Langerhans cells are easily identified in the mucosal epithelium. The inability of Weiss et al. to find Langerhans cells in the newborn may be explained by the fusion of the preputial mucosa to the glandular mucosa in the sterile intrauterine environment. Thus, Langerhans cells would not be expected in the fused prepuce/glans penile mucosa and may not be expected to appear until later in life when the prepuce becomes retractable and the mucosa is exposed to antigens (12).

Because of these anatomical, histological and immunological changes to the preputium, we hypothesized that preputial flora vary with age. Hence, we cultured the preputial area in uncircumcised boys of varying ages.

MATERIALS AND METHODS

This study was approved by our institutional review board and informed consent was obtained from the parents of all subjects. Between June 1 and November 30, 2002, prepuce and urine cultures were taken simultaneously from 92 uncircumcised and healthy male children aged between 0-12 years. Preputial swabs (PSs) were taken after the prepuce was retracted gently, and no external cleansing was performed before sampling. One sterile culture swab (Dio-Swab; Diomed, Istanbul, Turkey) was taken from each child by the same investigator and pressed circumferentially round the surface of the glans. The sample was immediately inserted in Dio-Transport swab/Stuart medium (Diomed) and sent to the laboratory where 5% sheep blood agar, eosin-methylene blue (EMB) agar and Sabouraud dextrose agar were inoculated, and the samples were incubated for 24-48 h. After the incubation period, the results were evaluated. Organisms were identified by classical microbiological methods and the API System (BioMerieux, Marcy l'Etoile, France).

After the preputial culture, the meatus was washed with soapy water and a midstream, clean-catch urine specimen was collected for culture. In young infants, urine samples were obtained using a sterilized urine bag attached to the penis. The urine samples were inoculated onto 5% sheep blood agar

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and EMB agar plates with a calibrated loop. The results were quantitatively reported after 24-48 h of incubation. Growth of greater than 10^5 CFU/ml of a single pathogen was considered to be significant depending on the organism and the clinical characteristics of the patient. When questionable, a second urine culture was obtained by suprapubic bladder aspiration. The same microbiologist reviewed all plates and isolates. Data were evaluated for significance using chi-square and Fisher's exact analysis.

RESULTS

Ninety-two healthy male children were divided into two groups according to age. Those 0-6 years were defined as Group A ($n = 47$), and those 7-12 years of age were Group B ($n = 45$).

In Group A, 43/47 (91.5%) of preputial cultures were positive (Table 1), and enteric bacteria were the most commonly isolated microorganisms from the prepuce ($\chi^2 = 9.18$, $P = 0.002$) (Tables 1, 2). Group A was then divided into subgroups according to their use of diapers (all children aged 0-2 years were using diapers), with no change in the results ($\chi^2 = 0.12$, $P = 0.52$). *Escherichia coli* was the most frequently isolated bacterium in those less under 2 years of age, while enterococci were predominant in those aged 3-6 years. Although *E. coli* ($n = 3$) and enterococci ($n = 1$) spp. were isolated from the urine cultures obtained by sterilized urine bags from 4 children, no bacteria were isolated in the specimens obtained by suprapubic bladder aspiration. We believe, therefore, that the positive urine cultures contained contaminants.

In Group B, 33/45 (73.3%) of the preputial cultures

were positive (Table 1); this was significantly less than in Group A ($\chi^2 = 5.27$, $P = 0.02$). Skin flora bacteria such as *Staphylococcus aureus*, and group A beta-hemolytic streptococci were the most common bacteria isolated from PSs in Group B (Tables 1, 2). Microbial growth was not observed in any of the urine samples from Group B.

DISCUSSION

It has been demonstrated that the preputial sac can act as a reservoir of organisms and is thus responsible for causing ascending UTIs (13-15). Wiswell et al. have shown that the peak concentration of uropathogenic organisms around the prepuce occurs at the time of life at which UTIs are most common in males (15). Many studies have shown that the preputial sac is heavily colonized with *E. coli* and other gastrointestinal flora during the first few years of life (13, 15-17). In the present study, *E. coli* was found to be the predominant microorganism in the preputial cultures of children between ages 0-2 years. Bollgren and Winberg have shown that the number of colonizing bacteria that can be potential uropathogens decreases rapidly with increasing age and in children above age 5, virtually no Gram-negative organisms were found in their study (5). In children 3-6 years old, we found a predominance of enterococci, and in those aged 7-12, there was a predominance of skin flora bacteria such as *S. aureus* and group A beta-hemolytic streptococci. Our results are thus consistent with those of Bollgren and Winberg (5).

Why are potential uropathogens diminished in older children in the periurethral and preputial areas? Several authors have written about the predominance of enteric bacteria in the preputial sac in the first years of life. According to these authors, contamination by enteric flora is especially likely to occur while using diapers; but as the child grows and acquires control of the anal and bladder sphincters, fecal contamination is reduced. This is also the time when personal hygiene begins to gain importance. Furthermore, changes in the retractability of the prepuce and the immune system may play a role (5,15,18). However, one study has claimed that there is no difference between a retractile and non-retractile prepuce with respect to bacterial flora between ages 2 and 12 (4). This may be better addressed by comparing cultures from circumcised and uncircumcised subjects.

In the present study, we found a significantly higher rate of sterile preputial cultures in children older than 6 years of age than in younger children. This is consistent with the findings of Cascio et al. (13) and Savas et al. (17) who have also reported that there was no bacterial growth in the periurethral or preputial sac in some of their patients. According to the study by Savas et al., 15.6-22.5% of preputial sac cultures were sterile in study groups that consisted of healthy children between the ages of 1 month and 8 years. Cascio et al. also have reported that 63% of subjects showed no bacterial growth in boys with vesicoureteral reflux (mean age 2.5 years) and 72.2% in boys undergoing circumcision for different medical reasons (mean age 5.1 years). However, none of these authors used sterile preputial cultures in their studies. These results are interesting and may be attributable to immune system maturation as well as to improved personal hygiene with age. ELCs would not be expected in the fused prepuce/glans penile mucosa and may not appear until later in life when the prepuce becomes retractable and the mucosa is exposed to antigens (12). Additionally, smegma

Table 1. Distribution of microorganisms isolated from PS samples according to ages

	0-6 y (Group A)		7-12 y (Group B)	
	n	%	n	%
Microbial growth (+)	43	91.5	33	73.3
Enteric flora	36	76.6	17	37.7
Skin flora	7	14.9	16	35.5
Microbial growth (-)	4	8.5	12	26.7
Total	47	100.0	45	100.0

PS, preputial swab.

Table 2. Distribution of microorganisms isolated from PS cultures

Isolated microorganisms	0-6 y (Group A)		7-12 y (Group B)
	0-2 y (n = 25)	3-6 y (n = 22)	(n = 45)
<i>Escherichia coli</i>	9	3	1
<i>Enterococcus faecalis</i>	2	10	9
<i>Staphylococcus aureus</i>	2	2	11
<i>Proteus mirabilis</i>	2	3	5
<i>Klebsiella pneumoniae</i>	3	1	1
<i>Enterobacter cloaca</i>	1	-	-
GABHS	-	-	5
<i>Candida albicans</i>	2	-	-
<i>Pseudomonas aeruginosa</i>	1	-	1
<i>Citrobacter freundii</i>	-	1	-
CNS	-	1	-
Total	22	21	33

GABHS, group A beta-hemolytic streptococci.

CNS, coagulase negative staphylococci.

plays an important role in the immune mechanism. Smegma is the compilation of seminal secretions from the Cowper's gland, the prostate and the urethral glands, as well as from the Tyson's glands. Cathepsin B, lysozyme, chymotrypsin, neutrophil elastase and cytokines present in the composition of smegma play an important role in the immune mechanism. While these secretions have an antimicrobial effect, they are not active in early childhood (19-21). We believe that the failure to detect bacterial growth in the PS culture of some patients is primarily related to immune system maturation.

E. coli ($n = 3$) and enterococci ($n = 1$) spp. were isolated from urine cultures, having been obtained by sterilized urine bag. No bacteria were isolated in second urine samples from the same subjects obtained by suprapubic bladder aspiration, confirming that cultures of urine specimens collected in a bag applied to the perineum have a significant false-positive rate (22).

The present study confirms that preputial flora change with age, showing a predominance of enteric bacteria in the first 6 years of life, while in older children, the bacterial content of the preputium resembles normal skin flora. This change is most likely related to the development of better immune status and to improvements in personal hygiene. For this reason we would encourage future studies on the composition of smegma, since it seems to play an important antibacterial and immunological role. An improved understanding of this process might alter medical recommendations regarding the use of circumcision and the age at which it should be performed.

ACKNOWLEDGMENTS

These data were presented as a poster with presentation category in the ESPU-AAP meeting in Uppsala, Sweden, 15-18 June 2005.

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