

## ORIGINAL ARTICLE

# Preputial Bacterial Pattern in Children With Hypospadias: Study From Skin Preparation Before Surgical Repair

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## ABSTRACT

**Introduction:** Surgical infection after hypospadias repair is still a problem affecting patient outcomes. Several factors, including bacterial invasion, skin preparation, cleansing method, and choice of antibiotic administration, may contribute to the development of infection. This study aimed to identify the pattern of preputial bacteria and their susceptibility to antibiotics in hypospadias patients. **Methods:** A study was carried out at the Pediatric Surgery Division Dr. Hasan Sadikin General Hospital, Bandung, from January 2017 to May 2021. A total of 58 hypospadias patients underwent preputial swab for bacterial culture and antibiotic susceptibility test. **Results:** The most common bacteria found in the preputial swab of hypospadias patients were *Staphylococcus hemolyticus* (34.84%), *Enterococcus faecalis* (10.6%), *Eschericia coli* (6.06%), *Morganella morganii* (6.06%), *Klebsiella pneumoniae* (4.54%), and *Staphylococcus warneri* (4.54%). Most of the bacteria showed a good sensitivity to the antibiotics tested. Five and one multidrug and extensively-drug resistant pathogens were found, respectively. Distal hypospadias showed the statistically significant predominance of *Staphylococcus* colonies, whereas gram-negative and mixed colonies were usually found in proximal hypospadias ( $P < 0.05$ ). **Conclusion:** Gram-positive bacteria were the most common pathogen found in preputial swab of hypospadias patients, and showed sensitivity toward quinolones.

**Keywords:** Bacteria, Drug Resistance, Hypospadias

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## INTRODUCTION

Hypospadias is a common birth defect of the male external genitalia. The prevalence of hypospadias has shown widespread variation geographically across the world, and it increases from year to year (1). The prevalence rate of hypospadias in Indonesia lacks certainty; studies conducted in several institutions in Indonesia showed hypospadias cases in the range of 10–17 cases per year (2). The number of cases was greater in our institution, given that hypospadias cases are common; we observed 147 cases of hypospadias in a three year period (3). If left uncorrected, hypospadias can cause several problems, such as psychological disorders due to penile shape, abnormal urine stream, and having to urinate in a sitting position. Hypospadias also causes reproductive problems in adult men (4). Although numerous studies have been conducted on hypospadias management, the complication rate and postoperative morbidity are still high, and the complications that often occur include

bleeding, edema, wound infection, and fistulas.

Several factors influence the incidence of postoperative complications, and they comprise the disruption of vascularization, high humidity and temperature, and the distance between the meatus opening and the area that can potentially contaminate intraoperatively. In addition, the use of dressings and the methods and materials used to clean postoperative wounds affect the incidence of postoperative complications. Apart from the information above, other factors during the preoperative period, such as prepuce and glans separation, cleaning of the epithelial debris in that area, and the lack of prophylactic antibiotics dose, can affect the incidence of complications, especially infection (5). External genital area usually turns pathogenic if the surrounding tissue is infected after surgery (6).

Infection plays an important role in the occurrence of other complications, such as wound dehiscence, urethra cutaneous fistula, crippled hypospadias, and difficult correction surgery. Local infection in surgical wounds can occur due to pre-existing bacterial colonization (7). Research on pathogens that cause infection has been conducted in India and revealed that *Coliforms* and

*Staphylococcus*, which are sensitive to cephalosporins and aminoglycosides, are the most common pathogens (8). In other studies by Rattan et al., the most common bacteria found were *Pseudomonas aeruginosa* and *Klebsiella spp.* (9). Antimicrobial resistance is now a major challenge for treating patients. Hence, this study was performed to detect the incidence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria isolated from hypospadias patients.

This study aimed to observe the bacterial patterns found in the prepuce of hypospadias patients treated in our institution along with their sensitivity to antibiotics. These data are important as baseline information to determine the subsequent management to reduce the incidence of complications after hypospadias surgery.

## MATERIALS AND METHODS

This research is a retrospective descriptive study of hypospadias patients admitted to the Pediatric Surgery Division from January 2017 to May 2021. This study was approved by Research Ethics Committee, Faculty of Medicine Padjadjaran University No. LB.02.01/X.6.5/295/2021 and Dr Hasan Sadikin General Hospital Bandung. Permission was also granted by the Head of Pediatric Surgery Division.

Out of 129 hypospadias patients during study time frame, 58 were recruited to the study based on the study inclusion criteria, which was hypospadias children who underwent preoperative cleansing with chloroxylenon solution and had a complete data of preputial swab examination. Data were collected from medical records and included clinical findings, and bacteria found from preputial swab along with their sensitivity to antibiotics, which were tested based on group A antibiotics that are recommended as appropriate for routine sensitivity tests from the Clinical Laboratory and Standard Institute. The bacteria that showed sensitivity to antibiotics were denoted as susceptible (S) (10). International standard definitions for acquired resistance by ECDC and CDC were used as definitions for MDR, XDR and PDR bacteria. MDR bacteria were defined to have acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. XDR bacteria were defined as those with nonsusceptibility to at least one agent in all but two or fewer antimicrobial categories (bacteria isolated remain susceptible to only one or two antimicrobial categories). PDR were defined as those with nonsusceptibility to all agents in all antimicrobial categories (11). The patients were also classified as distal and proximal hypospadias based on the location of external urethral meatus and were evaluated with the findings on the bacteria (6).

The frequencies of categorical data were tabulated, and the association of hypospadias severity and bacterial

pattern was analyzed using Fisher test ( $p < 0.05 =$  significant). Statistical analysis was performed using SPSS Version 17 software.

## RESULTS

The results of this study showed that the most common gram-positive bacteria found was *Staphylococcus hemolyticus* (35.84%), and the most common gram-negative bacteria found were *Enterococcus faecalis* (10.6%), *Eschericia coli* (6.96%), *Morganella morganii* (6.06%), *Klebsiella pneumoniae* (4.54%), *Staphylococcus aureus* (4.54%), *Staphylococcus warneri* (4.54%), and *Staphylococcus hominis* (4.54%) (Table I). Table I also shows other bacteria found in this study.

**Table I: Bacteria isolated from preputial swab**

Bacteria	Distal hypospadias	Proximal hypospadias	Frequency	%
<b>Staphylococcus</b>				
<i>S. hemolyticus</i>	5	18	23	34.84
<i>S. aureus</i>	1	2	3	4.54
<i>S. warneri</i>	0	3	3	4.54
<i>S. hominis</i>	3	0	3	4.54
<i>S. epidermidis</i>	2	0	2	3.03
<i>S. sciuri</i>	0	1	1	1.51
<b>Streptococcus agalactiae</b>	0	1	1	1.51
<b>Enterobacteriaceae</b>				
<i>Eschericia coli</i>	0	4	4	6.06
<i>Morganella morganii</i>	0	4	4	6.06
<i>Klebsiella pneumonia</i>	0	3	3	4.54
<i>Klebsiella oxytoca</i>	0	1	1	1.51
<i>Proteus Mirabilis</i>	0	2	2	3.03
<i>Citrobacter amalonaticus</i>	0	1	1	1.51
<b>Enterococcus</b>				
<i>Enterococcus faecalis</i>	0	7	7	10.6
<i>Enterococcus avium</i>	0	1	1	1.51
<b>Non-Enterobacteriaceae</b>				
<i>Pseudomonas stutzeri</i>	0	1	1	1.51
<i>Acinetobacter baumannii</i>	0	2	2	3.03
<i>Acinetobacter junii</i>	0	1	1	1.51
<i>Acinetobacter Iwofii</i>	0	2	2	3.03
<i>Commamonas testironi</i>	0	1	1	1.51
<b>Total</b>	<b>11</b>	<b>55</b>	<b>66</b>	<b>100</b>

We also observed that the bacteria isolated from patients (*Staphylococcus*) with more distal located meatus, showed uniformity. The culture of the results on 11 patients with distal located meatus (six subcoronal, three glanular, and two distal penile), showed five colonies of *S. hemolyticus*, three colonies of *S. hominis*, two colonies of *S. epidermidis*, and one colony of *S. aureus*. Significant differences in bacterial species were observed among distal meatus hypospadias patients and proximal hypospadias (RR: 9.47, 95% confidence

interval: 1.29–69.29,  $P < 0.05$ ) (Table II). The proximal located meatus showed a variety of bacteria from gram-positive to gram-negative bacteria.

**Table II. Bacteria according to severity of hypospadias**

Type of hypospadias	Gram positive bacteria		Gram negative bacteria		Total	P value
	n	%	n	%		
Proximal hypospadias	25	37.8	30	45.5	55	0.001
Distal hypospadias	11	16.7	0	0	11	
<b>Total</b>	<b>36</b>	<b>54.5</b>	<b>30</b>	<b>45.5</b>	<b>66</b>	

RR: 9.47, 95% CI 1.29-69.29

Antibiotic susceptibility test was carried out on all the bacteria isolated from the patients' prepuce. Figure 1 shows that all *Staphylococcus* were sensitive to gentamicin, levofloxacin, moxifloxacin, tigecycline and vancomycin. Other antibiotics with high sensitivity were trimethoprim-sulfamethoxazole and erythromycin. One isolated colony of *Streptococcus agalactiae* showed sensitivity to all the antibiotics tested.

Figure 2 shows that *Enterobacteriaceae* were most sensitive to amikacin. Other antibiotics that showed excellent sensitivity were gentamicin, tigecycline, ceftazidime and meropenem. Among all the *Enterobacteriaceae* bacteria, the colonies of *E. coli* showed poor sensitivity to the antibiotics tested. The *Enterococcus faecalis* colonies found in this study were entirely resistant to erythromycin and showed good sensitivity to almost all other antibiotics tested, such as those in Figure 3. The *Enterococcus avium* found in this study showed sensitivity to all the antibiotics tested.

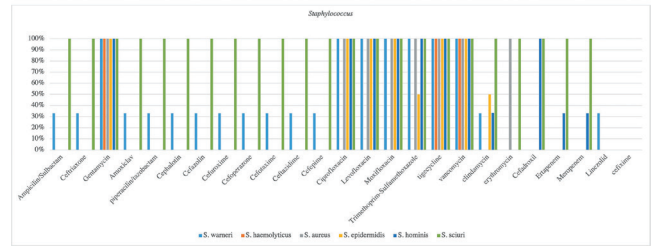
*Acinetobacter baumannii*, *Acinetobacter junii*, and *Comamonas testironi* found in this study were all sensitive to all the antibiotics tested. *Pseudomonas stutzeri* was resistant to piperacillin–tazobactam only (Figure 4).

Table III shows the incidence of the isolated MDR and XDR bacteria. Out of 66 bacteria isolated, 5 (7.5%) were MDR, and 1 (1.51%) was XDR. The MDR and XDR pathogens were from *Enterobacteriaceae* group, and neither MDR nor XDR strain was isolated from *Staphylococcus*, *Acinetobacter spp.*, and *Enterococcus spp.*

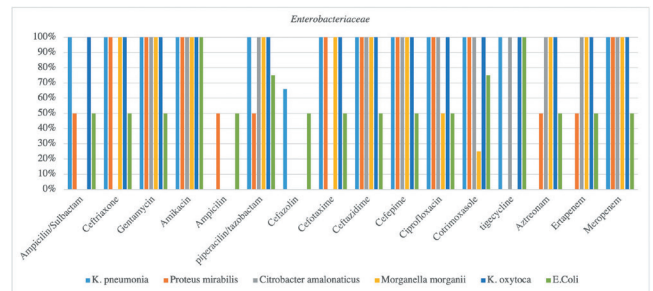
Patients were then followed up until 4 weeks post surgery, six patients (10.3%) reported signs of infections in less than 7 days post surgical. Eight patients (13.8%) had developed fistulae.

**DISCUSSION**

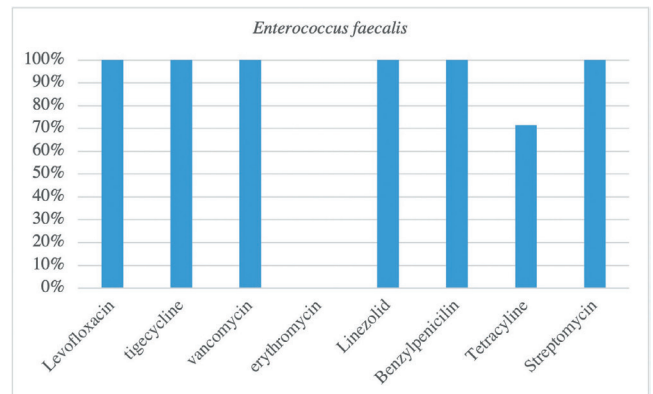
Early studies of the bacterial flora in the penis confirmed that the bacterial colonization of the



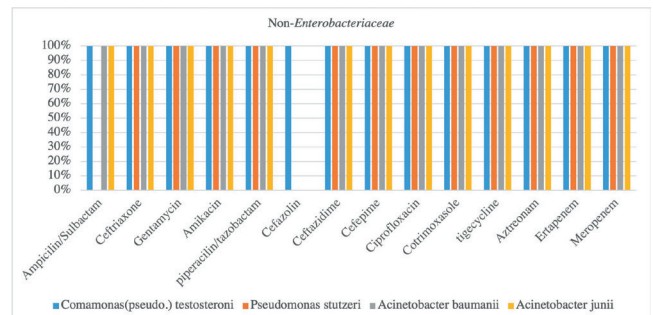
**Figure 1: Susceptibility of *Staphylococcus* to Antibiotics.** *Staphylococcus* showed sensitivity to gentamicin, levofloxacin, moxifloxacin, tigecycline, and vancomycin and high sensitivity toward trimethoprim-sulfamethoxazole and erythromycin. One isolated colony of *Streptococcus agalactiae* showed sensitivity to all the antibiotics tested.



**Figure 2: Susceptibility of *Enterobacteriaceae* to Antibiotics.** *Enterobacteriaceae* were most sensitive to amikacin. Other antibiotics showed excellent sensitivity were gentamicin, tigecycline, ceftazidime and meropenem. Among all the *Enterobacteriaceae* bacteria, the colonies of *E. coli* showed poor sensitivity to the antibiotics tested.



**Figure 3: Susceptibility of *Enterococcus faecalis* to Antibiotics.** *Enterococcus faecalis* colonies found in this study were entirely resistant to erythromycin and showed good sensitivity to almost all other antibiotics tested.



**Figure 4: Susceptibility of *Acinetobacter spp.* and *Pseudomonas stutzeri* to Antibiotics.** *Acinetobacter baumannii*, *Acinetobacter junii*, and *Comamonas testironi* found in this study were sensitive to all the antibiotics tested. *Pseudomonas stutzeri* showed resistance to piperacillin–tazobactam only.

**Table III: Incidence of MDR and XDR amongst bacteria isolated in this study**

Microorganism	MDR	XDR	PDR	Total
<i>S. aureus</i>	0	0	0	0
<i>Acinetobacter spp.</i>	0	0	0	0
<i>Enterococcus spp.</i>	0	0	0	0
<i>Enterobacteriaceae</i>	5	1	0	6
<b>Total</b>	<b>5</b>	<b>1</b>	<b>0</b>	<b>6</b>

prepuce is common (7). Spaine et al. reported that the most common bacterium found in normal penile was *Staphylococcus coagulase-negative* (12). The study conducted by Sanders et al. in hypospadias patients showed that the most common bacteria often found on the prepuce were *Enterococcus spp.* and *E. coli*, which is slightly in contrast to our results which showed the most isolated bacteria were *S. hemolyticus*, followed by *Enterococcus spp.*; however, we also found *E. coli* in our study (7). Another study conducted in Beijing showed that the most common bacteria that colonized the penis of hypospadias patients was *S. epidermidis*, which is different from our results; the authors also detected *Enterococcus spp.*, *P. mirabilis*, and *E. coli*, and the same bacteria were found in our study (13). In Indonesia, no studies have been conducted regarding bacterial patterns in hypospadias patients.

*Staphylococcus* and *Enterococcus spp.* were the most common bacteria found in this study, and both showed good sensitivity to quinolone and aminoglycoside antibiotics, while showing resistance to the cephalosporin antibiotic group, contrary to the results of the study conducted by Ratan et al., in which the colonies of *Staphylococcus* and *Coliforms* found still showed excellent sensitivity to cephalosporin antibiotics. Du et al. observed that *Staphylococcus* bacteria showed high rates of antibiotic resistance.

In recent years, strains of MDR organisms have quadrupled worldwide. Antimicrobial resistance is a major threat to antibiotic treatment (14). From this study we observed different number of MDR and XDR pathogens isolated from hypospadias patients compared with the prevalence reported in other studies worldwide. The findings on bacterial colonies on the prepuce, apart from normal flora that colonize the area, can also be caused by other factors, such as distance of meatus to the anus, were also found in this study. The further location of meatus from the anus (distal located meatus) showed the predominance of *Staphylococcus* colonization. Meanwhile, the more proximal location of meatus nearer to the anus showed variation in bacterial colonization, including gram-negative bacteria, such as *Enterococcus faecalis*, *E. coli*, and other bacteria, with statistically significant difference ( $P < 0.05$ ). Other factors related to the finding of bacterial colonies were poor personal hygiene, environmental temperature and high humidity from the environment around the patient

(8).

Data from this study are novel findings that can be useful in applications in our practice. However, this study showed several limitations, such as the preputial swab which was performed only once before surgery. In studies that have been conducted on hypospadias patients to evaluate the pattern of bacteria obtained from the preputial swab at different times, the first swab was performed preoperatively or before the surgical scrub, and the next swab was performed during intraoperative and postoperative wound swabs (after the first surgical wound dressing care) (8). This study did not examine the dressing type, duration of stent and examination of post-operative colonization. Another limitation was that this research is a single-center study. Thus, it could not reflect the trends of infection caused by MDR, XDR, and PDR pathogens. In future studies examinations should be carried out to compare the patterns of bacteria isolated preoperatively with those isolated from the postoperative to prevent postoperative wound infection, assess the effectiveness of the methods and materials used in prepuce cleansing during intraoperative procedures, and establish guidelines in the selection of postoperative antibiotics. A multicenter study should also be conducted to detect the incidence of MDR, XDR, and PDR organisms.

## CONCLUSION

The most common bacteria found from preputial swab consisted of gram-positive aerobic bacterium *S. hemolyticus*, followed by the gram-negative bacteria *Enterococcus faecalis*, *E. coli*, and *Morganella morganii*, with good sensitivity to quinolone and aminoglycoside antibiotics. *Staphylococcus* showed predominance in the distal hypospadias. Data regarding pattern of bacteria from the prepuce of hypospadias patients and their sensitivity to antibiotics, can provide guidance on antibiotic therapy in hypospadias patients who are treated in our institution.

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