Actinotignum schaalii Can Be an Uropathogen of “Culture-Negative” Febrile Urinary Tract Infections in Children with Urinary Tract Abnormalities

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Abstract
Accurate diagnosis and treatment of febrile urinary tract infections (UTI) during childhood are important for the prevention of renal parenchymal damage and functional loss, and detection of underlying diseases related to chronic kidney disease (CKD). Actinotignum schaalii (A. schaalii)-related febrile UTI in children is rare, and its incidence and risk factors remain unclear. A 3-year-old boy with a history of UTI presented with fever and vomiting. Although the culture of his urine specimen in air was negative, A. schaalii was observed in a 5% carbon dioxide (CO₂) culture condition, as well as an anaerobic one. A diagnosis of febrile UTI was made, and he recovered with antibiotic therapy. He was found to have CKD associated with vesicoureteral reflux (VUR) after further investigations. A. schaalii is one of the causative agents of febrile UTI in children with urinary tract abnormalities. Although the culture in the air could show negative results, urine culture in 5% CO₂ and anaerobic conditions is useful for diagnosis. Our case is the youngest and the first known case of A. schaalii-related febrile UTI associated with VUR in children.
Introduction

Accurate diagnosis and treatment of febrile urinary tract infections (UTI) during childhood are important for the prevention of renal parenchymal damage and functional loss. In addition, febrile UTI can reveal underlying diseases related to chronic kidney disease (CKD) in children, most of which are congenital anomalies of the kidney and urinary tract [1]. Most congenital anomalies of the kidney and urinary tract are detectable by renal ultrasound screening, while vesicoureteral reflux (VUR) is sometimes not, since VUR does not always have renal morphological abnormalities [2].

*Actinotignum* (formerly known as *Actinobaculum* schaalii (*A. schaalii*)) is a small rod-shaped, non-motile, non-sporulating, facultative anaerobe that requires carbon dioxide (CO₂) for its growth [3]. *A. schaalii*-related febrile UTI in children is rare, and its incidence and risk factors remain unclear. We report the case of a 3-year-old boy with febrile UTI caused by *A. schaalii* who was found to have CKD associated with VUR.

Case Report/Case Presentation

A 3-year-old Asian boy presenting with fever and chills was admitted to our hospital. His prenatal ultrasonographic results were normal, and he did not have a history of acute kidney injury (AKI) in the neonatal period. He had a history of febrile UTI caused by *Escherichia coli* (*E. coli*) at 6 months of age. During his first febrile UTI episode, his creatinine level was 0.66 mg/dL, with an estimated glomerular filtration rate (eGFR) of 50 mL/min/1.73 m²; however, it was never re-checked due to an unexplained reason.

The patient’s height and body weight were 103 cm (+0.8 standard deviation) and 16.4 kg, respectively, and his Rohrer index was 150. Physical examination revealed a 40°C fever, a heart rate of 150 beats per minute, a respiratory rate of 40 breaths per minute, and a blood pressure of 108/72 mm Hg. He had frequent episodes of vomiting and abdominal pain during urination.

Peripheral blood examination revealed the following: white blood cell count, 19,700/μL (normal range: 5,500–15,500/μL) (93% neutrophils); platelets, 17,000/μL (normal range: 100,000–450,000/μL); hematocrit: 40.4% (normal range: 34–40%); C-reactive protein, 17.9 mg/dL (normal range: <0.2 mg/dL); creatinine, 0.77 mg/dL (normal range: 0.21–0.37 mg/dL); and eGFR, 50 mL/min/1.73 m². Renal ultrasonography showed the right and left renal length of 79 mm (+1.5 SD), 76 mm (+1.0 SD), respectively, and bilateral hydroureters were also revealed.

A centrifuged urine sample collected via a transurethral catheter showed small rod-shaped, non-sporulating Gram-positive bacteria and numerous leukocytes on Gram staining (shown in Fig. 1). There was no bacterial growth on 5% sheep blood agar (Nippon Becton Dickinson, Inc.), but *Actinotignum schaalii* was isolated from the urine sample using an anaerobic blood agar plate. The isolate was identified using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The patient was treated with intravenous ceftriaxone for 7 days, and he was discharged with no complications.

Fig. 1. A centrifuged urine sample collected via a transurethral catheter shows small rod-shaped, non-sporulating Gram-positive bacteria and numerous leukocytes on Gram staining (×1,000).
Tokyo, Japan) or MacConkey agar (Nippon Becton Dickinson) at 35°C in the air. However, on 5% sheep blood agar at 37°C in a 5% CO₂ environment, as well as on anaerobic 5% sheep blood agar (Nissui Pharmaceutical, Tokyo, Japan) at 35°C in an anaerobic condition, we observed 10⁵ colony-forming units per milliliter of Gram-positive rods identical to the microorganisms seen on Gram staining. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (VITEK-MSTM, Sysmex bioMérieux, Tokyo, Japan) identified the Gram-positive rods to be *A. schaalii*. The probability of identification was 99%. He was diagnosed with a febrile UTI caused by *A. schaalii*. The minimum inhibitory concentrations (MIC) of the antibiotics we administered were ampicillin: <0.06 μg/mL and cefotaxime: <0.06 μg/mL.

On admission, ampicillin and cefotaxime were administered; the chills, shivering, vomiting, and abdominal pain resolved in 24 h, while the fever subsided in 48 h. Follow-up urine culture in a 5% CO₂ atmosphere was negative. Antibiotic therapy was continued for 14 days. Voiding cystourethrography (VCUG) performed 1 month later detected grade IV VUR in the right ureter and grade II VUR in the left. Dimercaptosuccinic acid (DMSA) scintigraphy performed 3 months after the febrile UTI episode revealed scarring in the upper pole of the right and the upper and lower poles of the left kidney. His renal size was within a normal range and his eGFR remained at 50 mL/min/1.73 m²; thus, we diagnosed him as having CKD associated with VUR, which may have been damaging his kidneys since the fetal period. Hypo/dysplastic kidneys were denied for his normal renal sizes. Cohen cross-trigonal reimplantation was performed to avoid a relapse of UTI 4 months after discharge. The patient was free of infection recurrence for 7 months after the second febrile UTI episode and his creatinine level remained unchanged. As his physical development was good and he had no recurrent episodes of infection other than UTIs, we concluded that he did not have the disorders of immune function.

**Discussion/Conclusion**

We presented the youngest and the first known case of *A. schaalii*-related febrile UTI associated with VUR in children. The results of this case revealed two important clinical issues: *A. schaalii* can be a causative agent of febrile UTI in children with urinary tract abnormalities, and adding culture conditions would help to grow fastidious bacteria such as *A. schaalii* when the standard culture result is negative. Expanding culture conditions, which is sometimes called enhanced quantitative urine culture (EQUC) [4], is one of the most clinically significant methods to improve detection of etiologies contributing to febrile UTI in children, who may have undiagnosed CKD associated with VUR.

*A. schaalii* can be a causative agent of febrile UTI in children with urinary tract abnormalities. *A. schaalii* might harmlessly inhabit the urethra in younger children, considering that it was detected, using real-time polymerase chain reaction, in urine samples from 30% of children under 4 years of age without febrile UTI [5]. However, *A. schaalii* can be an opportunistic pathogen when it gains access to sterile kidneys. Only three cases of febrile UTI due to *A. schaalii* in children have been reported [6–8]. One report was of a 5-year-old boy with pyeloureteral junction obstruction [6], while the other was of a 17-year-old boy with a single kidney on hemodialysis [7]. Another one was of a 14-year-old girl with a giant ureterocele [8]. In adults, there have been many reports claiming *A. schaalii* often causes UTI in elderly individuals with urologic obstruction [9]. As assessed by the above information, we reached two hypotheses. First, urinary tract abnormalities with abnormal urinary flow may be related to the development of febrile UTI caused by *A. schaalii* in children. Second, the immunologic state may also contribute to it. Immune dysfunction associated with opportunistic infection can occur in the elderly [10] and in end-stage renal disease [11]. Younger children without the disorders of immune function may also be susceptible to unusual infectious diseases because toddlers are
still immunologically immature [12]. We propose that the elderly, those with end-stage renal disease, and toddlers are the possible risk group of A. schaalii-related febrile UTI due to their immunological background.

Adding culture conditions would help to grow fastidious bacteria such as A. schaalii, when the standard culture result is negative. The standard culture protocol in urinary tract infections is usually including incubation on 5% sheep blood and MacConkey agars in air at 35°C for 24 h [4], in which condition, the major uropathogens such as Escherichia coli easily grow. Especially, 5% sheep blood agar has a great capacity to diagnose febrile UTI, providing enrichment for the growth of less common uropathogens [4]. However, as some of the causative agents cannot be detected by the standard culture protocol [4], EQUC is sometimes useful for diagnosis. For instance, incubation in an increased CO₂ environment or anaerobic culture promotes the growth of fastidious bacteria [4], as seen in our case. Chocolate agar improves the recovery of fastidious bacteria such as Haemophilus spp [13]. We agree with the opinion that EQUC should be considered when individuals with UTI-like symptoms have “no growth” via standard urine culture and for use with individuals with recurrent UTI [4]. This method will help us to determine whether a detected microorganism is a true pathogen or an only resident of the normal microbiota. The clinical importance of EQUC is increased by limiting its use to cases where the aforementioned conditions are met, such as in our case.

In our case, we considered the Gram stain test to be useful for diagnosis. However, the utility of urine Gram staining for diagnosing febrile UTI in children has been controversial [14, 15]. In a study comparing the concordance rates of Gram stain and bacterial culture results of urine collected and centrifuged from children, the sensitivity of the Gram stain test was 97.3% and the specificity was 73.8%, indicating a low specificity [15]. However, the culture conditions were not described in that study; hence, if the organisms found via Gram staining were difficult to grow, they may not have been recovered well because of inappropriate culture conditions. Additional or amended culture conditions, depending on the results of the Gram staining, may increase the agreement between Gram staining and culture tests.

Severe renal scarring can occur after even a single episode of febrile UTI [16], so we should not fail to detect febrile UTIs in children. Of note, evidence suggests that culture-negative febrile UTI is not uncommon. The frequency of culture-negative febrile UTI diagnosed by contrast-enhanced CT scan or DMSA scintigraphy in children has been reported as 9–38% [17, 18]. The actual incidence rate of culture-negative febrile UTI cases is uncertain because patients who have febrile UTI with negative results on standard urine culture without radiological investigations may be registered as fever of unknown origin. A. schaalii could be an overlooked fastidious uropathogen associated with culture-negative UTIs, which need appropriate antibiotic treatment and careful follow-up.

In our case, VUR with CKD was found upon further investigation after recurrent febrile UTI. We concluded that his renal function had worsened due to parenchymal damage from VUR since before birth because his creatinine level was already abnormally high at the age of 6 months, without AKI episodes or other morphological renal abnormalities. Previous studies reported that this scenario generally occurs in boys [19]. Although his VUR was not found at his first febrile UTI because the renal ultrasound screening revealed normal, we were able to detect his CKD before complications occurred, such as short stature, anemia, and electrolyte abnormalities.

In the past, VCUG was performed in all cases of febrile UTI in children. However, recently, the use of VCUG for the first UTI in children has been limited. The guidelines from both the American Academy of Pediatrics and the National Institute for Health and Care Excellence recommend that VCUG should not be routinely performed for the first febrile UTI in children [20, 21]. On the other hand, the European Association of Urology still recommends that all children at age 0–2 years after the first febrile UTI should undergo VCUG [22], although many
pediatric urologists have expressed disagreement with this opinion [23]. There are some reasons for recent changes in indications for VCUG. First, most VURs can be expected to resolve spontaneously [24]. Second, there is scarce evidence that early detection and intervention of VUR can improve the functional prognosis of the kidney [25], while antibiotics prophylaxis and ureterocystoneostomy may reduce the likelihood of recurrent febrile UTIs [26]. Third, the incidence of CKD in children with VUR without morphological abnormalities that can be detected with ultrasonography such as kidney hypoplasia is significantly low [27]. Finally, many pediatric experts are emphasizing VCUG as a terrible and traumatic experience for children with radiation exposure [23]. However, it should be noted that in some cases, as in our case, a high-grade VUR with decreased renal function may be hidden behind the first febrile UTI. Therefore, we re-emphasize the importance of correctly diagnosing recurrent febrile UTI, which may lead to the subsequent detection of VUR.

Lastly, we propose ampicillin or cefotaxime as reasonable options for treating *A. schaalii* infections. Unfortunately, *A. schaalii* has no drug susceptibility breakpoints in the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), although we assessed the MIC values of the antibiotics we administered to be sufficiently low since our patient recovered well after treatment with ampicillin and cefotaxime. In addition, the previous reports confirmed that the MIC values of beta-lactams and cephems against most isolates of *A. schaalii* were low [28], and these drugs were clinically effective [7].

In conclusion, *A. schaalii* can be one of the causative agents of febrile UTI in children with urinary tract abnormalities, and adding culture conditions would help to grow fastidious bacteria such as *A. schaalii*, when the standard culture result is negative. If the standard culture is negative despite the presence of bacteria on Gram staining and if the patient has UTI-like symptoms or he has the past history of UTI, culture workup in a 5% CO$_2$ atmosphere and anaerobic culture should be performed. Further studies are needed to clarify the frequency and the relationship between urologic abnormalities and UTIs caused by *A. schaalii* in children.

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**Statement of Ethics**

Ethical approval was not required for this study in accordance with local or national guidelines. This is an anonymous case presentation of a child. Written informed consent was obtained from the patient’s parents for publication of this case report and any accompanying images.”

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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Author Contributions

Mami Washio developed the manuscript idea and drafted the first version. Nobutaka Harada did the data collection and figures. Nobutaka Harada and Daisuke Nishima participated in drafting of the discussion of the manuscript, and Megumi Takemoto took part in revising the article critically. Mami Washio, Nobutaka Harada, Daisuke Nishima, and Megumi Takemoto read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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