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Case report

Urinary Tract Infection Caused by *Haemophilus influenzae* – A Case Report

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Abstract

Haemophilus influenzae is primarily recognized as a respiratory pathogen, with its role in urinary tract infections (UTIs) being infrequently reported. However, the actual incidence of *H. influenzae*-associated UTIs remains uncertain due to the bacterium's inability to grow on standard urine culture media. We present a case of a 14-month-old infant diagnosed with a UTI caused by *H. influenzae*, which subsequently led to the identification of anatomical abnormalities within the urinary tract. The child was exhibiting clinical symptoms of UTI, with biochemical urine analysis revealing positivity for nitrites and leukocytes. *H. influenzae* was incidentally detected from the child's urine culture by satellite growth around contaminating staphylococcal colonies. Further clinical evaluation confirmed vesicoureteral reflux (VUR) grade 4 and left-sided reflux nephropathy. *H. influenzae* may be an underdiagnosed pathogen in pediatric UTIs, particularly in cases where standard cultures yield negative results and underlying urinary tract abnormalities are present.

Keywords: urinary tract infections, *Haemophilus influenzae*, children

Резюме

Haemophilus influenzae е известен предимно като респираторен патоген, като ролята му в инфекциите на пикочните пътища (ИПП) се съобщава рядко. Реалната честота на ИПП, свързани с *H. influenzae*, обаче остава неясна поради неспособността на бактерията да расте върху стандартни среди за култивиране на урина. Представяме случай на 14-месечно бебе, диагностицирано с ИПП, причинено от *H. influenzae*, което впоследствие доведе до идентифициране на анатомични аномалии в пикочните пътища. Детето проявяваше клинични симптоми на ИПП, като биохимичният анализ на урината установи положителен резултат за нитрити и левкоцити. *H. influenzae* беше случайно открит от културата на урината на детето чрез сателитен растеж около замърсяващи стафилококови колонии. По-нататъшна клинична оценка потвърди везикоуретерален рефлукс (ВУР) степен 4 и лявостранна рефлуксна нефропатия. *H. influenzae* може да е недостатъчно диагностициран патоген при педиатрични ИПП, особено в случаите, когато стандартните култури дават отрицателни резултати и са налице подлежащи аномалии на пикочните пътища.

Introduction

Haemophilus influenzae is a fastidious, Gram-negative, pleomorphic bacillus that commonly colonizes the upper respiratory tract. The species includes both non-encapsulated and encapsulated strains, classified into six serotypes (a-f), with type b (*H. influenzae* type b, or Hib) historically associated with invasive infections. Since the introduction of the Hib vaccine, less invasive strains have gained increasing clinical significance (Resman *et al.,* 2011; ECDC, 2018).

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Pediatric UTIs are predominantly caused by members of the *Enterobacteriaceae* family, particularly *Escherichia coli*, while *H. influenzae* is rarely implicated (Hansson *et al.,* 2007; Allard *et al.,* 2012). The low detection rate of *H. influenzae* in UTIs may be attributed to its specific nutritional requirements, which hinders its growth on conventional urine culture media. Here, we report a rare case of a UTI in a 14-month-old female infant caused by *H. influenzae*.

Case Report

The patient, a 14-month-old female, was born at term following an uneventful pregnancy. Antenatal ultrasonography revealed no anomalies, and the child was fully vaccinated according to the national immunization schedule.

During the first year of life, the patient experienced three febrile episodes with significantly elevated inflammatory markers: C-reactive protein (CRP) levels of 22.5 mg/L, 104.6 mg/L, and 172.4 mg/L, along with leukocyte counts of 18.5×10^9/L, 19.9×10^9/L, and 17×10^9/L, respectively. Each episode was accompanied by normal pulmonary findings, and urine testing was not performed.

At 14 months of age, the patient presented with irritability, fever (38.2°C), and abdominal pain. Urinalysis using dipstick (HUMAN Combina 13) was positive for nitrites and leukocytes (corresponding to ~500 leukocytes/μL). Following the collection of a urine specimen for microbiological culture using a sterile urine collection bag, empiric antibiotic therapy with cefixime was initiated.

Standard urine culture media, including Columbia agar with sheep blood and Brilliance UTI Clarity agar, were used for inoculation. Due to laboratory processing delays, incubation was extended to 48 hours. Subsequent examination revealed several staphylococcal colonies on blood agar, surrounded by satellite growth indicative of *Haemophilus* species (Fig. 1). Gram staining of these colonies confirmed Gram-negative pleomorphic bacilli.

Further phenotypic identification demonstrated a requirement for both X (hemin) and V (nicotinamide adenine dinucleotide, NAD) growth factors, confirming *H. influenzae*. Automated identification was performed using the Vitek 2 Compact system (NH Card, Lot Number: 2452334203) (Fig. 2).

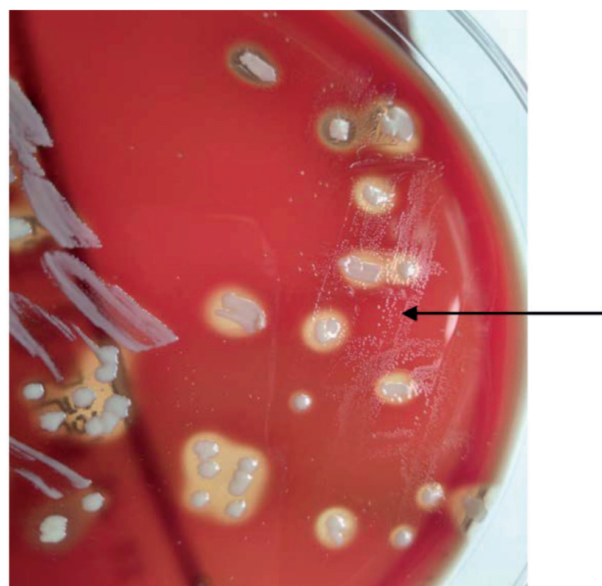

Fig. 1. *Haemophilus influenzae* colonies.
Arrow points to H. influenzae colonies - small, transparent, growing around colonies of *S. haemolyticus* (satellite phenomena)

bioMérieux Customer:
System #:
Patient Name:
Isolate: 887323-1 (Approved)
Card Type: NH Bar Code: 2452334203506298 Testing Instrument: 00000BAD80C8 (Micro)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: Labadmin
Patient ID: 887323

Bionumber: 1604040207
Organism Quantity:

Selected Organism: Haemophilus influenzae

Comments:	

Identification Information	**Card:** NH	**Lot Number:** 2452334203	**Expires:** Apr 17, 2024 13:00 CDT
	Status: Final	**Analysis Time:** 5.80 hours	**Completed:** Apr 11, 2023 19:31 CDT
Organism Origin	VITEK 2		
Selected Organism	85% Probability **Haemophilus influenzae** Bionumber: 1604040207 Confidence: Acceptable identification		

Analysis Organisms and Tests to Separate:

Analysis Messages:
See product information for additional information.

Contraindicating Typical Biopattern(s)
Haemophilus influenzae PheA(92),dMLT(88),dGAL(90),PHOS(99),

Biochemical Details

1	ArgA	+	2	GGT	-	3	LysA	-	4	dGAL	-	5	LeuA	+	6	ELLM	+
7	PheA	-	8	ProA	-	10	PyrA	-	13	TyrA	-	15	APPA	-	18	dGLU	+
19	GLYG	-	20	dMNE	-	22	dMAL	-	28	SAC	-	33	NAG	(-)	36	URE	+
39	BGALi	-	40	ODC	-	41	AARA	-	45	PVATE	-	46	PHC	+	47	dMLT	-
51	MTE	-	52	IGLM	-	59	PHOS	-	61	dRIB2	+	62	OPS	+	64	dXYL	+

Fig. 2. Automated identification of *H. influenzae* by Vitek 2 Compact system (NH Card, Lot Number: 2452334203)

bioMérieux Customer:
System #:
Patient Name:
Isolate: 10000323-1 (Approved)
Card Type: GP Bar Code: 2422088503763674 Testing Instrument: 00000BAD80C8 (Micro)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: Labadmin
Patient ID: 10000323

Bionumber: 030002047761271
Organism Quantity:

Selected Organism: Staphylococcus haemolyticus

Comments:	

Identification Information	**Card:** GP	**Lot Number:** 2422088503	**Expires:** Aug 15, 2023 13:00 CDT
	Status: Final	**Analysis Time:** 6.78 hours	**Completed:** Mar 29, 2023 18:11 CDT
Organism Origin	VITEK ?		
Selected Organism	94% Probability Bionumber: 030002047761271	Staphylococcus haemolyticus	Confidence: Very good identification

Analysis Organisms and Tests to Separate:

Analysis Messages:

Contraindicating Typical Biopattern(s)
Staphylococcus haemolyticus dMNE(1),BGAL(15),

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Fig. 3. Automated identification of *Staphylococcus haemolyticus*by Vitek 2 Compact system (GP Card, Lot Number: 2422088503)

Contaminating staphylococcal colonies were identified as *Staphylococcus haemolyticus* (GP Card, Lot Number: 2422088503) (Fig. 3).

Antimicrobial susceptibility testing, conducted using the Kirby-Bauer disc diffusion method and interpreted per EUCAST 2023 guidelines (EUCAST, 2023), revealed susceptibility to ampicillin, cefixime, ciprofloxacin, and trimethoprim-sulfamethoxazole. Amoxicillin-clavulanate was categorized as susceptible for intravenous administration and as susceptible with increased exposure for oral administration (Fig. 4).

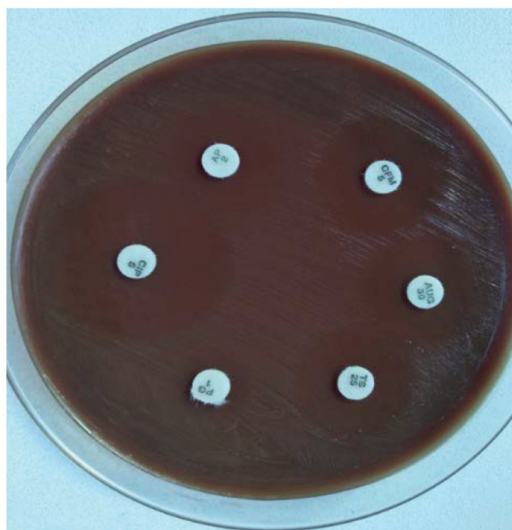

Fig. 4. Antimicrobial susceptibility test of the isolated *H. influenzae* strain from urine. (CIP-ciprofloxacin, AP-ampicillin, CFM- cefixime, AUG-amoxicillin-clavulanate, TS-trimethoprim-sulfamethoxazole, P-penicillin)

Given this unexpected microbiological finding, the patient was referred to the Pediatric Nephrology Department for further evaluation. Pyelonephritis due to *H. influenzae* was diagnosed, and treatment with cefixime was completed, followed by prophylactic antibiotic therapy.

Subsequent imaging studies included ultrasonography, which identified a dilated left ureter. A voiding cystourethrogram (VCUG) confirmed left-sided vesicoureteral reflux (VUR) grade 3-4. Renal scintigraphy using 99mTc-DMSA demonstrated an enlarged left kidney with cortical defects in the upper pole and superior lateral edge, indicative of reflux nephropathy (Fig. 5). The patient was diagnosed with left-sided VUR grade 4 and reflux nephropathy. At 2.5 years of age, she remains on prophylactic antibiotic therapy with nitrofurantoin suspension (2 mL nightly) and is in stable condition.

Discussion

To our knowledge, this is the first documented case of a pediatric UTI caused by *H. influenzae* in North Macedonia. The rarity of *H. influenzae*-associated UTIs may be attributed to its inability to grow on conventional urine culture media. Optimal growth requires enriched media such as brain heart infusion supplemented with hemin and NAD, or chocolate agar (Poje and Redfield, 2003). While

hemin is present in blood agar, NAD is sequestered within red blood cells. In this case, *Staphylococcus haemolyticus* likely provided the NAD necessary for *H. influenzae* satellite growth. Additionally, the prolonged incubation period enhanced the visibility of this phenomenon.

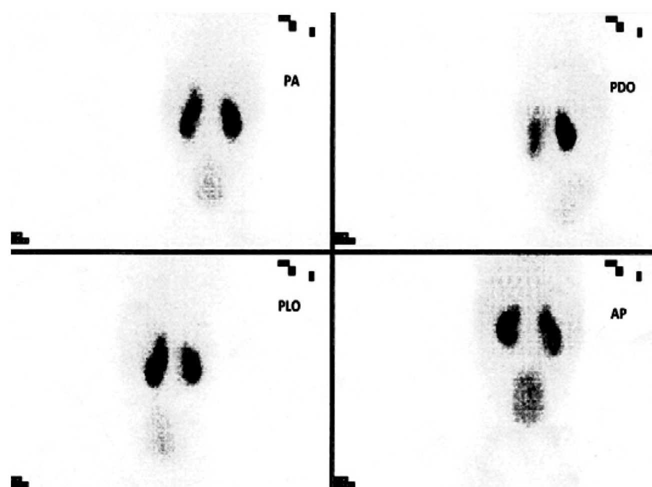

Fig. 5. Renal scintigraphy with 99mTc-DMSA. The right kidney is presented with a regular shape and size, homogeneously accumulating radiotracer, without cortical defects. The left kidney is larger with a cortical defect in the upper pole, as well as a defect in the projection of the superior lateral edge visible on PLO positioning.

Direct plating of the urine sample on chocolate agar for colony quantification was not feasible, as the original specimen had already been discarded. Furthermore, serotyping of the isolate could not be performed due to the unavailability of reagents. However, considering the child's complete vaccination history, the isolate was presumed to be a non-type b strain, consistent with current trends in *H. influenzae* epidemiology (ECDC, 2018; Bamberger *et al.,* 2014).

In terms of antimicrobial susceptibility, the isolation in this case exhibited full *in vitro* susceptibility to all tested agents, despite the increasing prevalence of antimicrobial resistance reported globally (Abavisani *et al.,* 2024).

In the present case, the detection of *H. influenzae*, an uncommon urinary pathogen, led to further clinical investigation, ultimately revealing structural abnormalities of the child's urinary tract. This observation aligns with existing literature, which describes *H. influenzae* as a rare cause of UTIs that typically occurs in the presence of underlying urinary tract abnormalities. A 24-year retrospective study identified *Haemophilus* spp. in only 36 of 5,000 pediatric UTI cases, with most of the affected children exhibiting renal abnormalities (Hansson *et al.*, 2007). Similarly, several case reports have linked *H. influenzae* pyelonephritis to structural uropathies (Allard *et al.*, 2012).

This case highlights the importance of considering UTI as a differential diagnosis in young children presenting with recurrent febrile episodes, especially in the absence of respiratory symptoms. Additionally, if a UTI is suspected in a child with known urinary tract dysfunction, urine cultures should include chocolate agar to enhance the recovery of *H. influenzae*. This underscores the need for close collaboration between clinicians and microbiologists to ensure accurate diagnosis.

In conclusion, further research is needed to determine the true incidence of *H. influenzae* UTIs and to develop optimized culture protocols that improve detection in pediatric populations.

Acknowledgments

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