ORIGINAL RESEARCH

Noninferiority of Multiplex Polymerase Chain Reaction Compared to Standard Urine Culture for Urinary Tract Infection Diagnosis in Pediatric Patients at Hackensack Meridian Health Children's Hospital Emergency Department

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Objective: To establish the noninferiority of the rapid and sensitive multiplex polymerase chain reaction (M-PCR) method versus standard urine culture (SUC) in pediatric urinary tract infection (UTI) diagnostic testing.

Methods: A United States of America (USA)-based single-center prospective observational study of 44 female and four male patients aged 3–21 years old presenting to a Pediatric Emergency Department in New Jersey with clinically suspected UTI. Urine specimens were primarily collected via midstream voiding. Patients with antibiotic exposure within the week prior to presentation were excluded. Patient demographic data, clinical manifestations, and urinalysis results were recorded. Noninferiority testing comparing M-PCR and SUC was conducted using a method for paired binary data, with a noninferiority margin set at 5%. Noninferiority was concluded if the lower bound of the 95% confidence interval of the difference in detection rates between M-PCR and SUC lies entirely to the right of the value minus the noninferiority margin. All statistical calculations were performed using Python 3.10.12.

Results: The two methods were concordant in two-thirds of cases. Of the 14 M-PCR-positive/SUC-negative discordant specimens, 13 (93%) contained a fastidious and/or emerging uropathogen (*A. urinae, A. schaalii, G. vaginalis, C. riegelii, U. urealyticum*, Viridans group Streptococci (VGS), and/or Coagulase-negative Staphylococci (CoNS)). Neither symptom presentation nor urinalysis results differed significantly between participants with concordant positive results for UTI diagnosis and those with concordant negative results (non-UTI group).

Conclusion: In this pediatric population, similar to previous findings in an older adult population, M-PCR established not only noninferiority but also superiority over SUC in detecting microorganisms in the urine.

Keywords: symptoms, urinalysis, UTI, SUC, M-PCR, Guidance UTI

Introduction

Pediatric urinary tract infection (UTI) constitutes a significant healthcare burden, leading to more than 1.1 million office visits and around 2.8% of children being diagnosed each year.[1,2] Furthermore, pediatric UTI can result in acute complications, like renal abscess, pyelonephritis, pyonephrosis, even urosepsis, as well as chronic kidney health complications such as renal scarring, hypertension, and/or insufficiency in adulthood.[3–10] Estimated costs are over $180 million annually for inpatient hospital care of children with pyelonephritis in the United States of America (USA) alone.[2] UTI recurrences in children are also common, especially in very young children and children with urinary tract abnormalities, primarily due to

unresolved bacteriuria due to inadequate antimicrobial treatment.[11] Therefore, prompt and accurate diagnosis and treatment of pediatric UTI are crucial to preventing UTI recurrence and potentially irreversible renal damage.

UTIs are caused by bacteria invading the kidneys, ureters, bladder, and/or urethra (which together constitute the urinary tract). Polymicrobial UTIs, which involve ≥2 species of microorganisms, have been considered rare. However, this assumption is related to the usual interpretation of standard urine culture (SUC), which is often dismissed as contaminated when more than one microbial species is present.[12–14] Yet, polymicrobial infections are being increasingly recognized as clinically relevant.[15–19] SUC also has inherent limitations, including a poor sensitivity for the detection of "emerging" or "opportunistic" uropathogens, which includes fastidious, anaerobic, slow-growing, and gram-positive microorganisms. Diagnosis by SUC is also far from the most time-efficient methodology, taking up to 3–5 days to provide bacterial identification and results of SUC with antimicrobial susceptibility testing.[20] Not all patients are systemically healthy enough to tolerate a time delay before treatment, which then results in clinical reliance on empiric antibiotic therapy.[17,21–23] Empiric antibiotic therapy, in turn, is associated with increased risk of treatment failure and developing antibiotic resistance.[21] Culture-based methods of diagnosis are therefore lacking not only in their sensitivity toward polymicrobial and non-*E. coli* infections but also in the speed necessary to provide optimal guided treatment for patients. Advanced molecular methods, including real-time multiplex polymerase chain reaction (M-PCR), offer faster and more sensitive UTI testing, with both organism identification/quantification and antibiotic susceptibility results available within 24 hours of urine specimen receipt in the laboratory.[24,25] Although M-PCR has demonstrated increased diagnostic sensitivity and improved clinical outcomes in older adults, evidence in pediatric populations is currently lacking. Therefore, this study was conducted to compare the performance of M-PCR and SUC in UTI diagnostic testing in pediatric patients.

Materials and Methods
Study Design
This USA-based, single-center, prospective observational study was conducted with the approval of the institutional IRB (Approval: Pro2021-0783). Patients 0–21 years old who presented to the Pediatric Emergency Department of a large academic children's hospital in New Jersey, USA, between 2/25/2022 and 10/22/2023, with suspected UTI based on physician assessment, and who were able to provide a urine specimen of sufficient volume via midstream voiding, catheterization, or wet diaper, were eligible. Patients treated with antibiotics within one week prior to presentation were excluded. Each participant's age, sex, and clinical presentation, including fever, abdominal pain, flank/back pain, nausea, vomiting, diarrhea, dysuria, malodorous urine, and/or altered urinary frequency, were documented.

Specimen Handling
Urine samples were immediately subjected to urinalysis and SUC protocols at the hospital clinical laboratory, according to the facility's standard procedures which follow the American Society for Microbiology Clinical Microbiology Procedures Handbook 5th Edition. The remaining urine sample was divided between a gray-top vacutainer (BD, Franklin Lakes, NJ, USA) containing boric acid and a yellow-top vacutainer, which does not contain a boric acid preservative. The vacutainers were labeled with a de-identified code and transported to Pathnostics via FedEx within the allotted specimen stability time of five days at room temperature. Once received, urine samples from the gray-top tubes were used for microbial identification by M-PCR, and samples from the yellow-top tubes were used for pooled antibiotic susceptibility testing (P-AST).

Bacterial Identification with Multiplex- Polymerase Chain Reaction (M-PCR)/Pooled Antibiotic Susceptibility Testing (P-AST)
The M-PCR was performed as previously described with two exceptions: 1) *Bacillus atrophaeus* served as the inhibition control and 2) probes and primers for detection of *Gardnerella vaginalis* were included in addition those listed in the references.[26,27] M-PCR is performed on DNA extracted from subject's urine samples. The microbial DNA is amplified using a universal PCR master mix and TaqMan technology on OpenArray chips.

Probes and primers were used to detect 30 microorganism species/groups and 32 antibiotic resistance genes (Supplemental Table S1). In addition to the organisms typically included in the M-PCR assay, this study also included probes and primers for microorganisms responsible for sexually transmitted infections, including *Chlamydia trachomatis, Mycobacterium tuberculosis, Neisseria gonorrhoeae, Proteus vulgaris*, and *Trichomonas vaginalis*, as well as for viruses such as the BK virus, Cytomegalovirus, Human Herpesvirus 6, Herpes Simplex Viruses 1 and 2, and JC virus.

Microorganism density was reported in semi-quantitative units of "<10,000", "10,000−49,999", "50,000−99,999", or "≥100,000" cells/mL, calculated from copies/mL. The cells/mL unit of quantification by M-PCR correlates linearly 1:1 with the colony forming units per milliliter (CFUs/mL) unit of quantification by SUC.[28] In this analysis, only samples with at least one microorganism detected at a density of ≥10,000 cells/mL were included. Although the traditional threshold for diagnostically significant microbial density in the USA is 100,000 cells/mL or CFUs/mL, our published data,[29–31] together with clinical guidances, suggest that a threshold of just 10,000 cells/mL or CFUs/mL may be more clinically relevant.[32,33] Antibiotic resistance gene detection was reported as a binary "detected" or "not detected" result.

P-AST is a component of the M-PCR/P-AST test which measures the phenotypic susceptibility of 19 UTI-related antibiotics against the entire "pool" of cultivable bacteria in a patient's urine specimen. P-AST is performed on all specimens in which M-PCR detects one or more non-fastidious bacterial species/groups. This "pooled" testing strategy is designed to account for interactions between multiple bacterial species that may change phenotypic resistance in polymicrobial infections. By combining genotypic resistance data from M-PCR with phenotypic susceptibility from P-AST, the M-PCR/P-AST assay provides clinicians with important actionable information for the management of complicated UTI cases. Since the P-AST result component is not relevant to organism identification, those results are outside the scope of this analysis.

Statistical Analyses

The confidence intervals of the agreement percentages between M-PCR and SUC were calculated using the Wilson score method. Symptom representation and urinalysis results between participants with concordant positive results versus concordant negative results were compared using the Fisher's exact test. Noninferiority testing comparing M-PCR and SUC was conducted using a method for paired binary data,[34] with a noninferiority margin set at 5%. Noninferiority was concluded if the lower bound of the 95% confidence interval of the difference in detection rates between M-PCR and SUC lies entirely to the right of the value minus the noninferiority margin. For all statistical tests, significance was defined as $p < 0.05$ and no adjustments were made for multiple comparisons. All statistical calculations were performed using Python 3.10.12.

Results

Participant Demographics and Symptoms

UTI diagnostic testing was performed on urine specimens from 44 females and four males. All but two specimens were collected via midstream voiding, with one collected using a catheter and one collected using a diaper. Participants ranged in age from 3 to 21 years, with a mean age of 12.3 years (Figure 1).

The most common symptom noted during clinical evaluation was dysuria, and approximately half (54%) of the participants presented with ≥2 of the specified symptoms (Table 1).

Urinalysis

All specimens underwent urinalysis, and most participants had abnormal urinalysis results. The most common finding was pyuria, with elevated white blood cell (WBC) count (92%) and/or positive leukocyte esterase (LE) (92%) (Table 2). More than half (56%) of the patients had hematuria, and 2/3 (67%) had proteinuria (Table 2). A few patients had elevated urine specific gravity (25%) or positive nitrite levels (23%) (Table 2).

Comparison of SUC and M-PCR Results

Overall, M-PCR yielded more positive results than SUC did (40 vs 28) (Table 3). The difference in detection rates was 25.0% (95% CI: 10.0–40.0). Because the lower bound of the CI was greater than the noninferiority margin (−5%),
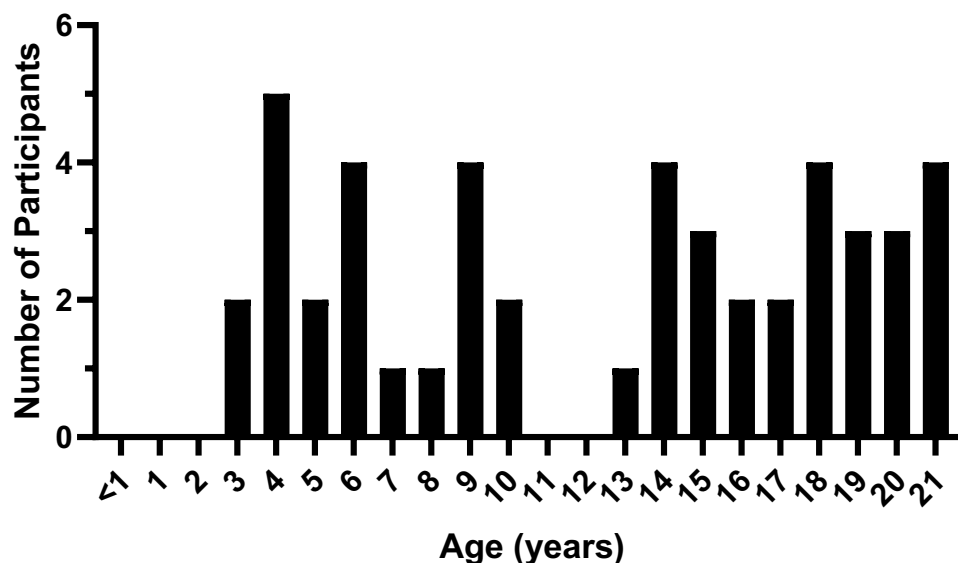
Figure 1 Distribution of Participant Ages. Age in years is plotted along the x-axis and data bars indicate the number of participants of that age along the y-axis.

noninferiority was demonstrated. Moreover, because the lower bound was greater than 0, the superiority of M-PCR over SUC in detecting pathogens was established. The two methods were two-thirds concordant (26 concordant positive and six concordant negative results) (Table 3). Of the 16 discordant results, 14 were M-PCR-positive/SUC-negative and only two were SUC-positive/M-PCR-negative (Table 3).

No SUC-positive specimens were reported to have polymicrobial results (≥2 individual species). M-PCR-positive specimens were 62.5% (n = 25) polymicrobial and 37.5% (n = 15) monomicrobial. Of the 14 M-PCR-positive/SUC-negative discordant specimens, 11 (79%) were polymicrobial, 13 (93%) contained a fastidious and/or emerging uropathogen (*A. urinae, A. schaalii, G. vaginalis, C. riegelii, U. urealyticum*, Viridans group *Streptococci* (VGS), and/ or Coagulase-negative *Staphylococci* (CoNS)), and all were from female participants (Table 4). In one case, *E. coli* was detected at a density of ≥10^5 cells/mL by M-PCR (equivalent to CFU/mL) but was reported as "negative" by SUC. Both

Table 1 Symptom Prevalence

Symptom	n	%
Dysuria	21	44
Abdominal Pain	19	40
Fever	10	21
Flank Pain	10	21
Vomiting	8	17
Altered Urinary Frequency	8	17
Malodorous Urine	2	4
Nausea	2	4
Diarrhea	1	2
Only One of these Symptoms	22	46
Multiple (≥2) of these Symptoms	26	54

Table 2 Urinalysis Results

Specific Gravity		n	%
Normal	1.05 to < 1.03	36	75
Abnormal	≥ 1.030	12	25
White Blood Cells (WBC)		n	%
Normal	0–5	4	8
	6–9	5	10
	10–19	6	13
	20–50	13	27
	>50	20	42
Red Blood Cells (RBC)		n	%
Normal	0–2	21	44
Abnormal	3–5	5	10
	6–9	4	8
	10–19	4	8
	20–50	8	17
	>50	6	13
Leukocyte esterase (LE)		n	%
Normal	negative	4	8
Abnormal	trace	3	6
	small	10	21
	moderate	16	33
	large	15	31
Nitrites		n	%
Normal	Negative	37	77
Abnormal	Positive	11	23
Protein		n	%
Normal	negative	7	15
Abnormal	trace	9	19
	30	18	38
	100	9	19
	300	5	10

SUC-positive/M-PCR-negative discordant specimens were obtained from male participants (Table 4). One was identified as *K. oxytoca* and the other as *S. maltophilia*, which is not targeted for detection by the M-PCR test (Table 4).

Neither symptom presentation nor urinalysis results differed significantly between participants with concordant positive results for UTI diagnosis and those with concordant negative results (non-UTI) (Table 5).

Table 3 SUC and M-PCR Concordance

	M-PCR Positive	M-PCR Negative	Total	Agreement	Wilson 95% CI
SUC Positive	26 (54%)	2 (4%)	28 (58%)	26/28 (92.9%)	(77.4%, 98.0%)
SUC Negative*	14 (29%)	6 (13%)	20 (42%)	6/20 (30.0%)	(14.5%, 51.9%)
Total	40 (83%)	8 (17%)	n = 48	(26+6)/48 (66.7%)	(52.5%, 78.3%)
Agreement	26/40 (65.5%)	6/8 (75%)	(26+6)/48 (66.7%)		
Wilson 95% CI	(49.5%, 77.9%)	(40.9%, 92.9%)	(52.5%, 78.3%)		

Notes: *Includes n = 14 "Negative", n = 2 "Contaminated specimens", n = 2 "non-significant flora", and n = 2 "Non-uropathogenic gram-positive organisms".

Table 4 Details of Discordant Results

SUC Result	M-PCR Result	Sex	Age
Negative	A.schaalii (>100,000 cells/mL)	F	4
	E. faecalis (>100,000 cells/mL)		
	E. coli (>100,000 cells/mL)		
Negative	E. coli (>100,000 cells/mL)	F	9
Non-uropathogenic gram-positive organism	CoNS (>100,000 cells/mL)	F	9
	G. vaginalis (>100,000 cells/mL)		
	E. coli (10,000–49,999 cells/mL)		
	S. aureus (10,000–49,999 cells/mL)		
Contaminated specimen	A. schaalii (10,000–49,999 cells/mL)	F	10
	CoNS (10,000–49,999 cells/mL)		
Non-uropathogenic gram-positive Organism	E. coli (>100,000 cells/mL)	F	10
	A. urinae (10,000–49,999 cells/mL)		
Non-significant flora	E. faecalis (>100,000 cells/mL)	F	13
	K. oxytoca (>100,000 cells/mL)		
	CoNS (10,000–49,999 cells/mL)		
Negative	CoNS (>100,000 cells/mL)	F	14
	VGS (>100,000 cells/mL)		
Negative	E. coli (>100,000 cells/mL)	F	15
	E. faecalis (>100,000 cells/mL)		
	G. vaginalis (50,000–100,000 cells/mL)		
	CoNS (10,000–49,999 cells/mL)		
	U. urealyticum (10,000–49,999 cells/mL)		
	C. albicans (10,000–49,999 cells/mL)		

(*Continued*)

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Table 4 (Continued).

SUC Result	M-PCR Result	Sex	Age
Negative	*G. vaginalis* (>100,000 cells/mL)	F	15
Non-significant flora	VGS (>100,000 cells/mL)	F	16
Contaminated specimen	*C. riegelii* (>100,000 cells/mL)	F	18
	CoNS (>100,000 cells/mL)		
	E. faecalis (>100,000 cells/mL)		
	E. coli (>100,000 cells/mL)		
	VGS (50,000–100,000 cells/mL)		
	A. schaalii (10,000–49,999 cells/mL)		
Negative	*E. coli* (>100,000 cells/mL)	F	19
	G. vaginalis (50,000–100,000 cells/mL)		
Negative	*C. koseri* (>100,000 cells/mL)	F	20
	K. pneumonia (>100,000 cells/mL)		
	G. vaginalis (10,000–49,999 cells/mL)		
	C. albicans (10,000–49,999 cells/mL)		
Negative	*G. vaginalis* (>100,000 cells/mL)	F	21
Klebsiella oxytoca	Negative	M	9
Stenotrophomonas maltophilia	Negative	M	9

Table 5 Comparison of Symptoms and Urinalysis Criteria Between UTI and Non-UTI Cases

Diagnostic Criterion		Concordant Positives (n = 26)		Concordant Negatives (n = 6)		*p-value*
		n	%	n	%	
Symptoms	Fever	6	23	2	33	0.625
	Abdominal Pain	9	35	1	17	0.637
	Flank Pain	8	31	1	17	0.648
	Nausea	1	4	0	0	1.000
	Vomiting	3	12	2	33	0.228
	Diarrhea	0	0	1	17	0.188
	Dysuria	14	54	2	33	0.654
	Malodorous Urine	1	4	0	0	1.000
	Altered Urinary Frequency	6	23	0	0	0.564

(*Continued*)

Table 5 (Continued).

Diagnostic Criterion		Concordant Positives (n = 26)		Concordant Negatives (n = 6)		*p-value*
		n	%	n	%	
Urinalysis	Specific Gravity	5	19	2	33	0.590
	Pyuria	24	92	6	100	1.000
	Hematuria	17	65	3	50	0.647
	Proteinuria	25	96	5	83	0.345
	+ Leukocyte esterase	24	92	5	83	0.476
	+ Nitrites	8	31	1	17	0.648

SUC detected gram-negative bacteria in 20 specimens, gram-positive bacteria in seven specimens, and yeast in one specimen, but did not detect fastidious bacteria in any specimen (Figure 2). M-PCR detected gram-negative bacteria in 35 specimens, gram-positive bacteria in 32 specimens, yeast in three specimens, and fastidious bacteria in 28 specimens (Figure 2). According to both the SUC (29%, n = 14) and M-PCR (50%, n = 24) results, *E. coli* was the most prevalent organism (Figure 2). The next most prevalent organisms according to the SUC results were *Staphylococcus* species other

Organism	Method	Cases		%		Organism	Method	Cases		%	
E. coli	SUC		14	29%		*E. faecalis*	SUC		1	2%	
	M-PCR		24	50%			M-PCR		8	17%	Gram -positive Bacteria
M. morganii	SUC		0	0%		VGS**	SUC		0	0%	
	M-PCR		2	4%			M-PCR		10	21%	
C. koseri	SUC		0	0%		*S. agalactiae* (Group B *Strep.*)	SUC		1	2%	
	M-PCR		1	2%	Gram-negative Bacteria		M-PCR		1	2%	
P. mirabilis	SUC		1	2%		CoNS***	SUC#		5	10%	
	M-PCR		3	6%			M-PCR		11	23%	
K. pneumoniae	SUC		0	0%		*S. aureus*	SUC		0	0%	
	M-PCR		2	4%			M-PCR		2	4%	
P. aeruginosa	SUC		0	0%		*A. urinae*	SUC		0	0%	
	M-PCR		1	2%			M-PCR		2	4%	Fastidious Bacteria
Enterobacter Group*	SUC		3	6%		*A. schaalii*	SUC		0	0%	
	M-PCR		1	2%			M-PCR		7	15%	
K. oxytoca	SUC		1	2%		*G. vaginalis*	SUC		0	0%	
	M-PCR		1	2%			M-PCR		15	31%	
*S. maltophilia^*	SUC		1	2%		*C. riegelii*	SUC		0	0%	
	M-PCR		NA	NA			M-PCR		1	2%	
C. albicans	SUC		1	2%	Yeast	*U. urealyticum*	SUC		0	0%	
	M-PCR		3	6%			M-PCR		3	6%	

Figure 2 Comparison of Organism Prevalences as Detected by SUC Versus M-PCR.
Notes: * Enterobacter Group includes *Klebsiella aerogenes* (formally *Enterobacter aerogenes*) and *Enterobacter cloacae*. ** VGS includes *Streptococcus anginosus, Streptococcus oralis*, and *Streptococcus pasteurianus*. *** CoNS includes *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis*, and *Staphylococcus saprophyticus*. # SUC results reported for *Staphylococcus* species other than *S. aureus* are included. ^ *Stenotrophomonas maltophilia* was not targeted for detection by the M-PCR assay.

than *S. aureus* (10%, n = 5) and *Enterobacter* group organisms, including *Klebsiella aerogenes* (6%, n = 3) (Figure 2). According to M-PCR results, the most prevalent organisms other than *E. coli* were *G. vaginalis* (31%, n = 15), CoNS (23%, n = 11), VGS (21%, n = 10), *E. faecalis* (17%, n = 8), and *A. schaalii* (15%, n = 7) (Figure 2). There were no major trends in prevalence with age or sex, except for *G. vaginalis*, which was only detected in females aged ≥9 years. There were no viruses or microorganisms associated with sexually transmitted infections detected by M-PCR.

Discussion

We compared SUC and M-PCR for UTI diagnostic testing in a pediatric population (≤21 years).

Symptoms and Urinalysis are Insufficient Diagnostic Tools

Approximately half of the participants presented with ≥2 of the specified symptoms, with dysuria being the most prevalent symptom. Most participants had positive urinalysis results, with the majority having pyuria, as evidenced by elevated WBC counts and/or positive LE. More than half of the patients had hematuria, and 2/3 had proteinuria, but relatively few had elevated urine-specific gravity or positive nitrites. Ultimately, no individual symptoms or urinalysis results differed significantly between participants with concordant positive results for UTI diagnosis and those with concordant negative results (non-UTI group). Consistent with previous reports,[35–37] these findings demonstrate that these indicators are likely to be individually insufficient for UTI diagnosis. However, this finding is unsurprising given that the signs and symptoms of UTI were the inclusion criteria for this study. Additionally, the concordant negative group "n" was small. Future studies utilizing a robust control group will be more appropriately powered to ascertain the utility of individual symptoms and urinalysis findings as well as combinations with the potential to differentiate UTI.

Comparison of SUC and M-PCR Detection Sensitivity

According to both SUC and M-PCR, *E. coli* is the most prevalent organism. Although the prevalence (50% of SUC-positive and 60% of M-PCR-positive detections) was considerably lower than the traditionally presumed prevalence of up to 95%,[38,39] it was consistent with the previously reported prevalence of *E. coli* in adult populations.[26,27,40–42]

SUC detected gram-negative bacteria in 42%, gram-positive bacteria in 15%, and yeast in 2% of specimens but did not detect fastidious bacteria in any specimen. M-PCR detected gram-negative bacteria in 73%, gram-positive bacteria in 67% of specimens, yeast in 6%, and fastidious bacteria in 58% of specimens. SUC detected exclusively non-*E. coli* infections in 29% of the specimens compared to 33% by M-PCR. These results are consistent with previous reports, showing that M-PCR is more sensitive than SUC, particularly for the detection of non-*E. coli* infection in older adults.[40,43–45]

No SUC-positive specimens were reported to have polymicrobial results (≥2 individual species). In contrast, 62.5% of M-PCR-positive specimens were polymicrobial, which is consistent with the rate of polymicrobial identification reported by M-PCR in urine specimens from adults aged ≥60 years with symptoms of UTI.[16,25,26,40,45–48] Few studies have examined the clinical impact of polymicrobial infections, particularly in infrequent circumstances when more than two or three individual species are identified. In adults, those treated according to M-PCR/P-AST results, particularly those with polymicrobial infections identified by M-PCR, had significantly better outcomes, indicating the clinical significance of accurately recognizing polymicrobial UTIs.[25,49] To the best of our knowledge, no studies on polymicrobial UTIs in pediatric populations have been conducted to date.

SUC and M-PCR Concordance

The results of the two methods were concordant for two-thirds of the specimens. Of the 16 discordant results, 14 were M-PCR-positive/SUC-negative. All but one of the 14 M-PCR-positive/SUC-negative discordant specimens were poly-microbial and/or contained a fastidious and/or emerging uropathogen (*A. urinae, A. schaalii, G. vaginalis, C. riegelii, U. urealyticum*, VGS, and/or CoNS), consistent with reports that M-PCR is more sensitive than SUC for the detection of non-*E. coli* or polymicrobial infections in older adults.[40,43–45] In the remaining case, *E. coli* was detected at a high density by M-PCR but was reported as "negative" by SUC. Unpublished P-AST results confirmed that *E. coli* from this

specimen were viable in culture. This case demonstrates that M-PCR can also detect classical gram-negative uropathogens missed by SUC, as previously reported.[50]

In contrast, two specimens had positive microbial identifications by SUC but not by M-PCR; highlighting methodological differences between the two approaches. Although SUC-based detection and identification is limited to organisms that can grow under specific culture conditions, M-PCR-based detection and identification are limited to organisms for which probes and primers are included in the assay. The solution to this limitation of M-PCR is to continually refine the assay targets for scientific understanding of the prevalence and clinical relevance of different organisms in the urinary tract.

Presence of Non-Classical Uropathogens

Fastidious emerging uropathogens[43,51] detected by M-PCR in this pediatric population included *A. urinae, A. schaalii, C. riegelii, U. urealyticum*, and *G. vaginalis*. Both *A. urinae* and *A. schaalii* have been primarily reported in urine specimens from geriatric UTI patients with case reports of severe complications, including bacteremia and infective endocarditis.[52–54] Despite the association with geriatric patients, case reports and series have previously identified both organisms in pediatric UTI.[55–58] Interestingly, although *A. urinae* has been associated with malodorous urine,[57] the two subjects who reported these symptoms were both found to have *E. coli* infections by M-PCR and/or SUC, and neither of the two subjects with *A. urinae* detected by M-PCR reported malodorous urine as a symptom. *C. riegelii* has previously been reported to be associated with UTI and urosepsis in adults,[59–61] whereas case reports of hyperammonemic encephalopathy resulting from *C. riegelii* UTI in pediatric patients support the importance of identifying this organism in children.[62,63] Although not previously associated with pediatric UTI, *U. urealyticum* has been associated with chronic unexplained urinary symptoms in adult women and invasive infections in immunocompromised individuals.[64,65] All three subjects with *U. urealyticum* detected in their urine had polymicrobial infections with to 4–6 individual species detected. Two of these polymicrobial infections contained *E. coli*, whereas the third did not contain any classical uropathogens. *G. vaginalis* was the only organism that exhibited trends in prevalence with age or sex and was only detected in females aged ≥9 years. This is consistent with reports that *G. vaginalis* has an approximately 30% prevalence in adolescent females and is more prevalent in the vaginal microbiome after the onset of menarche and sexual contact.[66,67] Although primarily considered a part of the vaginal microbiome, *G. vaginalis* can enter the urinary tract and, when detected, can be associated with recurrent UTI.[68–70] Additionally, VGS and CoNS, non-fastidious organisms, were detected. Traditionally considered contaminants of the urogenital microbiome,[15,71] these organisms can be found in catheter-collected urine specimens[41] and are becoming increasingly recognized as opportunistic uropathogens in both adults and children.[72–79]

Strengths, Limitations, and Future Directions

Existing studies on advanced molecular diagnostic testing for UTIs have focused primarily on high-risk adult populations. This study expands into the pediatric population and sets the stage for future studies to evaluate whether the higher sensitivity of M-PCR translates to improved clinical outcomes, as have been demonstrated in older adults.[25] Future studies will examine the impact of the improved sensitivity of M-PCR coupled with the rapid 24-hour turnaround time for pooled antibiotic susceptibility testing (P-AST) on clinical outcomes in the pediatric population. Such P-AST data, including the companion P-AST data from this study, will also facilitate the generation of pediatric-UTI-specific antibiograms. In addition to their clinical value in the selection of empiric antibiotic therapies, an understanding of such antibiograms provides population-specific epidemiological data[80,81] which informs antibiotic stewardship efforts.

The main limitation of this study is its single-site design, which resulted in a relatively small sample size. The study population was heavily skewed toward adolescent females and did not include infants under the age of three. Given that the infant population exhibits a unique epidemiology with a higher prevalence of UTI in male infants,[1] future studies specifically in this population are warranted. Furthermore, because the infant population presents additional diagnostic challenges owing to the vagueness of atypical symptom presentation and nonverbal symptom communication,[82] the evaluation of M-PCR in conjunction with diagnostic urine biomarkers of UTI[83] is a particularly promising direction for future studies.

Conclusion

In this pediatric population, similar to previous findings in an older adult population, M-PCR is more sensitive than SUC for UTI diagnosis. The two methods were concordant in two-thirds of the cases, with a difference in detection rates of 25.0% (95% CI: 10.0–40.0). Since the lower bound of the confidence interval was greater than 0, M-PCR established not only noninferiority but also superiority over SUC for detecting microorganisms in urine.

Abbreviations

CFU, colony forming unit; CoNS, Coagulase-negative Staphylococci; M-PCR, multiplex-polymerase chain reaction; P-AST, pooled-antibiotic susceptibility testing; SUC, standard urine culture; USA, United States of America; UTI, urinary tract infection; VGS, Viridans group Streptococci.

Data Sharing Statement

All relevant data are present within the manuscript text, figures, and tables, or are available upon request from the authors.

Ethics Approval and Consent to Participate

This study was a USA-based single-center prospective observational study conducted in accordance with the Declaration of Helsinki and with the approval of the Hackensack Meridian Health Network IRB institutional review board (HMHN IRB; Approval: Pro2021-0783). The HMHN IRB granted an exemption from the requirement of written informed consent (both by participants aged 18 years and older and by the parents/guardians of participants under 18 years of age) because the research was conducted on remnant specimens remaining after collection and use for routine clinical diagnostic testing.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Emery Haley, Natalie Luke, Mohit Mathur, Xiaofei Chen, Jim Havrilla, and David Baunoch, are employees of Pathnostics. Natalie Luke has patents US 10,160,991, US 11,053,532, US 63/514,785, US16/848,651, BR112019021943-9 B, and NZ 759292 issued to Pathnostics and patents US 17/178,091, US 17/335,767, US 17/830,227, US 18/351,385, US 18/351,286, US 63/493,416, US18/451,748, and US 63/493,416 pending to Pathnostics. David Baunoch holds stocks from Pathnostics, has patents US10,160,992, US11,053,532, US17/335/767, US11/747,371, AU2018254514 B2, BR112019021943-9 B1, CA 3,175,879, CA 3,176,586, CA 3,061,015, HK 62020014337.3, CN 201880039956.9, and IL 294577 issued to Pathnostics and patents US17/178,091, US17/830/227, US18/351,286, US18/669,370, PCT/US22/16816, PCT/US22/77477, NZ759292, EP3612638, and JP2022-042545 pending to Pathnostics. The other authors have no conflicts of interest to declare.

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