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ORIGINAL RESEARCH

The Prevalence and Association of Different Uropathogens Detected by M-PCR with Infection-Associated Urine Biomarkers in Urinary Tract Infections

Emery Haley[1], Natalie Luke[1], Mohit Mathur[2], Richard A Festa[3], Jimin Wang[4], Yan Jiang[4], Lori A Anderson[5], David Baunoch[3]

[1]Department of Clinical Research, Pathnostics, Irvine, CA, USA; [2]Department of Medical Affairs, Pathnostics, Irvine, CA, USA; [3]Department of Research and Development, Pathnostics, Irvine, CA, USA; [4]Department of Statistical Analysis, Stat4Ward, Pittsburgh, PA, USA; [5]L.Anderson Diagnostic Market Access Consulting, San Diego, CA, USA

Correspondence: David Baunoch, Pathnostics, 15545 Sand Canyon Suite 100, Irvine, CA, 92618, USA, Tel +1-714-966-1221, Fax +1-714-966-1231, Email dbaunoch@pathnostics.com

Background: Many emerging uropathogens are currently identified by multiplex polymerase chain reaction (M-PCR) in suspected UTI cases. Standard urine culture (SUC) has significantly lower detection rates, raising questions about whether these organisms are associated with UTIs and truly cause inflammation.

Objective: To determine if microbes detected by M-PCR were likely causative of UTI by measuring inflammatory biomarkers in the urine of symptomatic patients.

Design, Setting, and Participants: Midstream voided urine was collected from subjects ≥60 years presenting to urology clinics with symptoms of UTI (n = 1132) between 01/2023 and 05/2023. Microbe detection was by M-PCR and inflammation-associated biomarker (neutrophil gelatinase-associated lipocalin, interleukin 8, and interleukin 1β) was by enzyme-linked immunosorbent assay. Biomarker positivity was measured against individual and groups of organisms, *E. coli* and non-*E. coli* cases, emerging uropathogens, monomicrobial and polymicrobial cases.

Outcome Measurements and Statistical Analysis: Distributions were compared using 2-sample Wilcoxon Rank Sum test with 2-tailed p-values < 0.05 considered statistically significant.

Results and Limitations: M-PCR was positive in 823 (72.7%) specimens with 28 of 30 (93%) microorganisms/groups detected. Twenty-six of twenty-eight detected microorganisms/groups (93%) had ≥2 biomarkers positive in >66% of cases. Both non-*E. coli* cases and *E. coli* cases had significant biomarker positivity ($p < 0.05$). Limitations were that a few organisms had low prevalence making inferences about their individual significance difficult.

Conclusion: The majority of microorganisms identified by M-PCR were associated with active inflammation measured by biomarker positivity, indicating they are likely causative of UTIs in symptomatic patients. This includes emerging uropathogens frequently not detected by standard urine culture.

Plain Language Summary: The M-PCR assay is a novel diagnostic assay for UTI.

This study found that most organisms included in the M-PCR assay were:

- detected in the urine of patients at least 60 years of age with a presumptive UTI diagnosis
- associated with biomarkers of infection and inflammation

Thus, the M-PCR assay:

- is clinically relevant
- has a low likelihood of false-positivity for UTI

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Introduction

Urinary tract infections (UTIs) are infections of any part of the urinary tract, generally grouped into lower UTI, called cystitis, in which the infection is confined to the bladder, and upper UTI, called pyelonephritis, in which the infection has spread to the kidneys.[1] UTIs constitute a significant healthcare burden worldwide.[2] A diagnosis of UTI is a leading cause of prescribed antibiotic usage in outpatients,[3] with most infections being treated empirically. Most UTIs occur in otherwise healthy, sexually active, young adult females, in whom anatomic and lifestyle factors result in a predisposition to cystitis.[4] However, while simple UTIs, particularly cystitis, are typically managed successfully with empirically prescribed antibiotics in an outpatient setting, patients with additional risk factors often require guided treatment. Newborns, children, elderly adults, and persons with diabetes or other comorbidities are at increased risk for recurrent and/or complicated UTIs (r/cUTIs).[4,5] These groups, particularly elderly adults, have higher treatment failure rates and poorer outcomes, such as UTI recurrence,[6] urosepsis,[7] and even death).[8] In 2018 r/cUTIs accounted for approximately >600,000 hospitalizations at an estimated mean cost of $70,063 per hospitalization (non-CAUTI related) in the US.[9] As the threat of microbial antibiotic resistance continues to increase, providing the correct antibiotic treatment quickly enough to avoid prolonged empiric therapy is a growing concern among healthcare stakeholders.[10–13]

As a diagnostic test for UTI, standard urine culture (SUC) has been in use for over 60 years with little advancement to accommodate for the identification of more recently discovered emerging uropathogens.[14] The standard urine culture method is optimized for the growth of gram-negative bacteria, primarily *Escherichia coli (E. coli*), the most commonly identified organism in acute UTIs.[15,16] Furthermore, the turn-around time for SUC, which includes antimicrobial susceptiblity testing, can be 3–5 days, potentially delaying results-guided antimicrobial treatment even in cases where the causative organism is detected.[15] Recent studies have shown that when more sensitive culture techniques such as enhanced-quantitative urine culture (EQUC) are used, many additional clinically relevant microbial species including several gram-positive organisms, fastidious microbes, and fungi have been isolated from symptomatic subjects.[17]

Similarly, previous studies have demonstrated that multiplex-PCR (M-PCR) is superior for detecting non-*E. coli* and polymicrobial infections in urine specimens compared to SUC.[12,13,18,19] Polymicrobial infections, which have been reported in up to 39% of suspected UTI cases in older adult populations,[17,20,21] have specifically been associated with poorer outcomes.[22] Additionally, M-PCR has the benefit of faster turnaround times to reported results, allowing for a more rapid transition to directed antimicrobial therapy or avoiding empiric therapy altogether.[12,13,18,19]

Despite these advantages, the clinical validity of identifying additional organisms by M-PCR in the urine of symptomatic subjects with UTIs has been questioned in terms of relevance to causing UTIs. Recently, the urine biomarkers neutrophil gelatinase-associated lipocalin (NGAL), interleukin 8 (IL-8), and interleukin 1 beta (IL-1β) have demonstrated a positive correlation and high specificity for active UTI infections.[23–27] These biomarkers become elevated in urine as resident and recruited immune cells rapidly mount a pro-inflammatory response to pathogens detected within the urinary tract.[28,29]

The purpose of this study was to validate the relevance of individual microbial species or groups using three infection-associated biomarkers, NGAL, IL-1β, and IL-8, as an indicator of the state of the immune system in conjunction with a unique M-PCR assay for detection and quantification of microorganisms in patients with lower urinary tract symptoms and diagnosed presumptively with UTIs in a specialty setting.

Materials and Methods

Study Design

This study utilized banked urine specimens from a randomly collected cross-section of 1132 subjects, at least 60 years old, presenting at urology clinics in 22 US states between 01/17/2023 and 05/16/2023 with clinical presentations consistent with UTI, and for which there was enough specimen to effectively conduct M-PCR and biomarker studies. The samples included in the biobank and used for this analysis are intended to be representative of the samples that

Table 1 Biomarker Positivity Cutoffs

Biomarker	Cutoff
Neutrophil gelatinase-associated lipocalin (NGAL)	≥ 38.0 ng/mL
Interleukin 8 (IL-8)	≥ 20.6 pg/mL
Interleukin 1 beta (IL-1β)	≥ 12.4 pg/mL

would routinely be sent for urine microorganism identification and quantification testing as part of the diagnosis and management of cases seen in outpatient urologic specialty settings. Since this study utilized urine samples from a biobank in which the samples were de-identified and associated only with the assigned ICD-10-CM code(s) and the subject's age and sex, the study was exempted from review from the Western Institutional Review Board- Copernicus Group (WCG), an external independent agency that reviews and approves industry-sponsored clinical trials.

All urine samples utilized in this study were collected via the midstream voided "clean catch" method which is standard practice for busy clinical offices. Samples were transferred to gray-top boric acid (for M-PCR) and yellow-top (for P-AST and biomarker analysis) Vacutainer Tubes (Becton Dickinson, Franklin Lakes, NJ) and shipped overnight at ambient temperature for evaluation at a central testing laboratory (Pathnostics, Irvine CA). Urine samples were processed for M-PCR/P-AST and for urinary biomarkers (NGAL, IL-1β, and IL-8) by enzyme-linked immunosorbent assay (ELISA). Only samples where microbes were detected above a positivity threshold ≥10,000 cells/mL for bacteria/bacterial groups and >0 cells/mL for yeasts by M-PCR were included in the biomarker analysis.

Specimen Testing

Biomarker Quantitation by ELISA – ELISAs for NGAL, (human Lipocalin-2/NGAL Quantikine ELISA Kit (Catalog number SLCN20), human IL-1β/IL-1F2 Quantikine ELISA kit (Catalog number SLB50), and human IL-8/CXCL8 Quantikine ELISA Kit (Catalog number S8000C), purchased from R&D Systems/Bio-Techne (Minneapolis, MN) were performed using the manufacturer's instructions-for-use with a TECAN microplate reader (Infinite M Nano+) taking OD measurement readings at 450nm and 540nm. Biomarker positivity was defined by using threshold values previously published (Table 1).[30,31] This study defined biomarker consensus as any combination of at least two of the three biomarkers positive at or above the cutoff levels.

Multiplex- Polymerase Chain Reaction (M-PCR) and Pooled Antibiotic Susceptibility Testing (P-AST) – The M-PCR/P-AST assay (Guidance® UTI, Pathnostics, Irvine, CA) analyzes 27 individual uropathogens, three bacterial groups, 32 antibiotic-resistance genes, phenotypic Extended-Spectrum Beta-Lactamase (ESBL), and pooled phenotypic susceptibility testing against 19 antibiotics. It is intended for use as a diagnostic test in symptomatic patients suspected of having active complicated, persistent, recurrent, and elevated-risk urinary tract infections. Testing was performed as previously described; however, results of antibiotic resistance gene detection, ESBL phenotype, and P-AST were not considered in this study.[12,13,32]

Statistical Analysis

Participant demographics and ICD-10-CM code breakdown were described by summary statistics (eg, mean and standard deviation (SD) for continuous variables such as age, number, and percentage for categorical variables such as sex and ICD-10-CM). Summary statistics (n, median, mean) of all three biomarker levels were provided. Among all M-PCR positive cases, the number and percentage of cases positive for biomarkers and consensus biomarkers were listed for each of the organisms and for combinations of the organisms. Statistical comparisons of biomarkers were compared using subgroup median values via the Wilcoxon test. All hypothesis tests were 2-sided, and a p-value < 0.05 was considered statistically significant. All data analyses were performed using R 4.2.2 (https://www.r-project.org/).

Results

Subject Demographics

The study included 1132 subjects presenting to urology clinics with symptoms of r/cUTI. The median subject age was 76.3 (range 60.0–103 years), and the mean was 76.6 (standard deviation = 8.72). Female patients comprised the majority

Table 2 Demographics of the Study Cohort

Demographics	
Age	
Mean (SD)	76.6 (8.72)
Median [Min, Max]	76.3 [60.0, 103]
Sex	
Female	752 (66.4%)
Male	380 (33.6%)
Total	1132

of the cohort, 66.4% (n = 752), and males accounted for 33.6% (n = 380) (Table 2). Many specimens were associated with 2 or more ICD-10-CM (https://www.icd10data.com) codes. The most prevalent of these ICD-10-CM codes was N39.0 "Urinary tract infection, site not specified" [76.0% (n = 977)]; followed by R30.0 "Dysuria" [8.1% (n = 104)]; R31.0 "Gross hematuria" [3.3% (n = 42)]; Z87.440 for "Personal history of urinary (tract) infections" [1.8% (n = 23)]; and R31.9 for "Hematuria, unspecified" [1.2% (n = 16)]. All other r/cUTI-related ICD-10-CM codes, each with a prevalence of <1% of subjects, were grouped under "Other" (Supplemental Table S1).

Microbial Prevalence with Detection and Identification by M-PCR/P-AST Assay

All 1132 specimens were tested for the presence of microbes by M-PCR; of those, 823 (72.7%) were positive. Within these positive specimens, M-PCR identified 1589 microorganisms, with a significant fraction of the total cases being polymicrobial infections (n = 522, 46.1%). Of the 27 species and three groups of microorganisms included in the M-PCR assay, only two (*A. baumannii* and *P. agglomerans*) were not detected in any specimen (Figure 1). Two-thirds of the microorganisms (20 of 30) accounted for approximately 99% of all positive results at the case level (Supplemental Table S4).

We analyzed the levels of biomarkers based on the classification groups of the detected microorganisms. The list of classifications and references is provided in Supplemental Table S2. Among the top five most prevalent organisms, we observed a diverse representation: one belonged to the classical gram-negative category (*E. coli*), one to the classical gram-positive type (*E. faecalis*), and three belonged to the emerging and/or fastidious uropathogen group (*A. urinae, A. schaalii*, and Viridans Group *Streptococcus* [VGS]). Gram-negative bacteria were detected in 581 (51.3%) specimens with over half of those (57.8%, n = 336) identified as *E. coli*. Gram-positive bacteria were detected in 438 (38.7%) specimens, of which 40.4% (n = 177) were identified as *E. faecalis*. Fastidious organisms were detected in 570 (50.4%) of total cases. *A. urinae* was the predominant species identified in 224 (39.3%) cases with fastidious organisms detected. Yeasts were detected in 40 cases (3.5%), and *C. glabrata* accounted for over half of the detected yeasts (n = 22, 55%). Additionally, we found that two organisms traditionally considered contaminants from the skin, VGS [(n = 160), 14.1% and Coagulase Negative *Staphylococcus* (CoNS) [(n = 49), 4.3%], were among the top 10 most prevalent organisms detected in the study specimens.

Infection-Associated Biomarkers in M-PCR-Positive Urine Samples

In order to comprehensively assess the presence of infection-associated biomarkers (NGAL, IL-8, and IL-1β), we analyzed the same urine specimens in which microorganisms were detected by M-PCR. By comparing biomarker positivity based on the thresholds outlined in Table 1, we examined the rate of biomarker positivity among different groups of organisms. In Table 3, the 30 detectable microorganisms are presented in groups of 5, by descending order of prevalence, starting with the five most frequently detected organisms, followed by the next most prevalent 5, and ending with the five organisms detected with the least frequency.

Urine samples with detected organisms exhibited high percentages of biomarker positivity. Specifically, NGAL showed a positivity rate of 81%, IL-8 showed a positivity rate of 86%, and IL-1β exhibited a positivity rate of 64%. Furthermore, the simultaneous positivity rate of two or more biomarkers was observed in 80% of cases. To provide

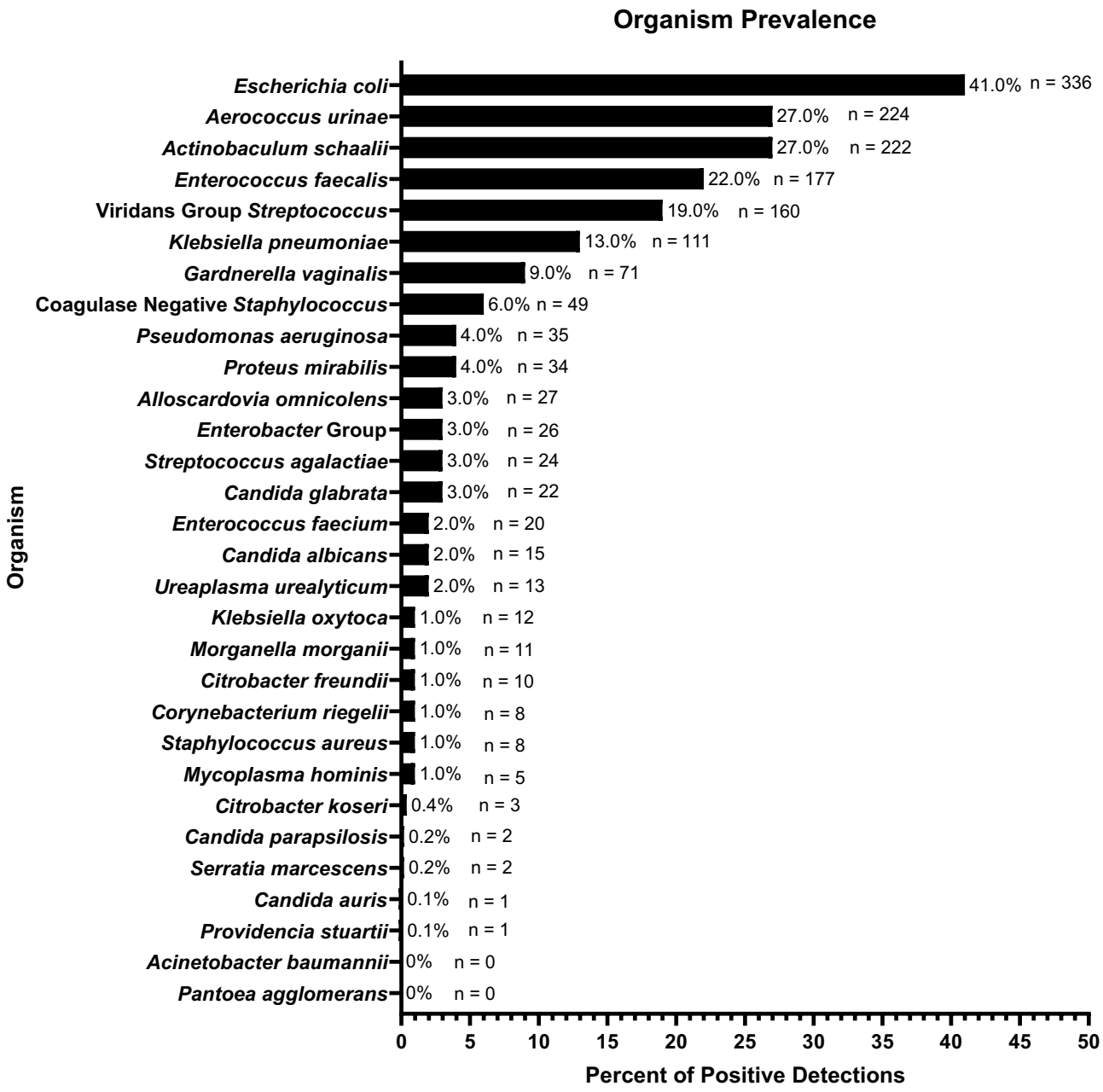

Figure 1 Organism detection prevalence.
Note: Organisms or organism groups are arranged on the y-axis in descending order of detection prevalence. Bar length along the x-axis represents the percent of M-PCR-positive specimens (microbial density ≥ 10,000 cells/mL for bacteria/bacterial groups or > 0 cells/mL for yeasts). The number (n) of detections are shown with labels at the end of each bar.

a more granular analysis, we delved into the biomarker positivity rates for each individual organism (refer to Supplemental Table S3) and sub-grouped the organisms starting with the top five most detected organisms, gradually expanding in groups of five (refer to Supplemental Table S4).

Considering the remarkable sensitivity of M-PCR in detecting a diverse array of organisms extending beyond *E. coli* and classical uropathogens,[12,13,18,19] we examined the biomarker positivity in all (both positive and negative) M-PCR specimens (Table 4, Supplemental Table S5A) and stratified cases into different groups (Table 5, Supplemental Table S5B). These groups comprised cases with and without *E. coli* detection, cases with solely classical uropathogens detected, and cases exhibiting exclusively emerging uropathogens (Table 5, Supplemental Table S5B). Furthermore,

Table 3 Biomarker Positivity in Groups of Five Organisms by Prevalence

Organisms as Detected at Density ≥ 10,000 by M-PCR, in Order of Prevalence	M-PCR Positivity	Individual and Consensus Biomarker Positivity Among All M-PCR-Positive Cases			
	n (%) of M-PCR Positive Cases	Consensus n (%) of M-PCR Positive Cases	NGAL n (%) of M-PCR Positive Cases	IL-8 n (%) of M-PCR Positive Cases	IL-1β n (%) of M-PCR Positive Cases
Overall M-PCR Positive Cases	823 (100%)	661 (80%)	670 (81%)	709 (86%)	529 (64%)
5 Most Prevalent Organisms (*Escherichia coli, Aerococcus urinae, Actinobaculum schaalii, Enterococcus faecalis*, Viridans Group *Streptococcus*)	648 (79%)	517 (80%)	526 (81%)	556 (86%)	415 (64%)
Next 5 (*Klebsiella pneumoniae, Gardnerella vaginalis*, Coagulase Negative *Staphylococcus, Pseudomonas aeruginosa, Proteus mirabilis*)	272 (33%)	226 (83%)	228 (84%)	240 (88%)	181 (67%)
Next 5 (*Alloscardovia omnicolens, Enterobacter* group, *Streptococcus agalactiae, Candida glabrata, Enterococcus faecium*)	117 (14%)	98 (84%)	98 (84%)	101 (86%)	74 (63%)
Next 5 (*Candida albicans, Ureaplasma urealyticum, Klebsiella oxytoca, Morganella morganii, Citrobacter freundii*)	60 (7%)	54 (90%)	54 (90%)	54 (90%)	46 (77%)
Next 5 (*Corynebacterium riegelii, Staphylococcus aureus, Mycoplasma hominis, Citrobacter koseri, Candida parapsilosis*)	26 (3%)	24 (92%)	25 (96%)	24 (92%)	18 (69%)
5 Least Prevalent Organisms (*Serratia marcescens, Candida auris, Providencia stuartii, Acinetobacter baumannii, Pantoea agglomerans*)	4 (<1%)	2 (50%)	2 (50%)	4 (100%)	2 (50%)

Notes: Biomarker positivity is presented by the total number and percentage of multiplex polymerase chain reaction (M-PCR) positives for each microbe-prevalence group.

Table 4 Biomarker Positivity in M-PCR Positive versus Negative Cases

Total n = 1132	M-PCR		Consensus		NGAL		IL-8		IL-1β	
	n	% of Total	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative
M-PCR Negative cases	309	27	35% (108)		39% (119)		50% (156)		22% (67)	
M-PCR Positive cases	823	73	80% (661)	**<0.0001**	81% (670)	**<0.0001**	86% (709)	**<0.0001**	64% (529)	**<0.0001**

Note: Bolded values indicate $p < 0.05$.

Table 5 Biomarker Positivity in M-PCR Positive Cases Grouped by Organism and Infection Characteristics

Total n = 823	M-PCR		Consensus		NGAL		IL-8		IL-1β	
	n	% of Total	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative
Mono-microbial cases	301	37	82% (246)	**<0.0001**	81% (243)	**<0.0001**	89% (269)	**<0.0001**	67% (201)	**<0.0001**
Poly-microbial cases	522	63	80% (415)	**<0.0001**	82% (427)	**<0.0001**	84% (440)	**<0.0001**	63% (328)	**<0.0001**
Cases with *E. coli*	336	41	86% (289)	**<0.0001**	86% (289)	**<0.0001**	91% (305)	**<0.0001**	72% (243)	**<0.0001**
Cases without *E. coli*	487	59	76% (372)	**<0.0001**	78% (381)	**<0.0001**	83% (404)	**<0.0001**	59% (286)	**<0.0001**

(*Continued*)

Table 5 (Continued).

Total n = 823	M-PCR		Consensus		NGAL		IL-8		IL-1β	
	n	% of Total	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative	% Positive (n)	p-value vs Negative
Cases with only emerging uro-pathogens	109	13	74% (81)	**<0.0001**	83% (90)	**<0.0001**	78% (85)	**<0.0001**	55% (60)	**<0.0001**
Cases with only classical uro-pathogens	442	54	78% (345)	**<0.0001**	76% (337)	**<0.0001**	86% (379)	**<0.0001**	63% (280)	**<0.0001**

Note: Bolded values indicate $p < 0.05$.

given the substantial capability of M-PCR to identify a significantly higher number of polymicrobial infections we also compared biomarker levels between specimens with monomicrobial and polymicrobial samples (Table 5, Supplemental Table S5B).[12,13,18,19] Across all groups of M-PCR positive specimens, all three biomarkers (NGAL, IL-8, and IL-1β) had a significantly higher percent positivity ($p < 0.0001$) than in M-PCR negative cases.

We examined the biomarker consensus percent positivity for each subgroup (Table 5). All subgroups of M-PCR-positive specimens had a biomarker consensus positivity ≥70%, ranging from 74% for cases with only emerging uropathogens to 86% for cases with *E. coli* detected. The biomarker consensus positivity for each subgroup was also significantly higher than that of the M-PCR-negative cases ($p < 0.0001$).

We also examined the biomarker consensus positivity for individual organisms to confirm their status as uropathogenic organisms (Figure 2). Biomarker consensus positivity rates were independent of microorganism prevalence in the study cohort. For example, *Gardnerella vaginalis*, the seventh most prevalent microorganism detected (n = 71) had a 66% biomarker consensus percent positivity, while *Mycoplasma hominis*, detected in only 5 specimens, had 100% biomarker consensus percent positivity (Figure 2 and Supplemental Table 3). Of the detected organisms, all but 2 [*Providencia stuartii* (n = 1) and *Serratia marcescens* (n = 2)] had >66% consensus biomarker positivity. Overall, 80% (n = 661) of the 823 M-PCR-positive specimens were positive for biomarker consensus.

Discussion

To guide antimicrobial selection for UTI patients, clinicians currently rely on microbial identification and quantitation by SUC and associated antibiotic susceptibility testing. Failure to detect and correctly identify those organisms missed by SUC can result in many UTIs remaining undiagnosed and untreated or being sub-optimally treated with empiric broad-spectrum antibiotics which potentially prolongs symptoms or results in serious complications.[33] Though it is evident that M-PCR has greater sensitivity than SUC there are some questions about the value of detecting these organisms, and whether they are associated with UTIs or are incidental findings.

We found that of the 30 organisms/organism groups included in the assay for this study just two (*A. baumannii* and *P. agglomerans*) were not detected in these symptomatic presumed UTI cases, though those 2 organisms have previously been shown to be uropathogenic.[34–41] Additionally, we also found that two organisms traditionally considered contaminants from skin, VGS and CoNS, were among the top 10 most prevalent organisms detected in the study specimens.[42] Other studies identified VGS and CoNS in both midstream voided and catheter-collected specimens at similar prevalence and densities, further indicating the organisms' likely pathogenic nature.[43] Therefore, the approach of detecting only classical uropathogens may result in missed cases, as emerging or less common pathogens can cause infections and pose significant health threats.

Having shown that these organisms were found within a significant number of presumed UTI cases, we then examined their association with urinary biomarkers associated with UTIs. Biomarker percent positivity was significantly higher for all 3 biomarkers in M-PCR positive specimens, compared to M-PCR negatives overall ($p < 0.001$). The small number of M-PCR-negative specimens with elevated biomarkers may represent UTIs caused by viruses, yeast, or bacteria not targeted by the M-PCR test, or by non-infectious bladder inflammation, such as interstitial cystitis.

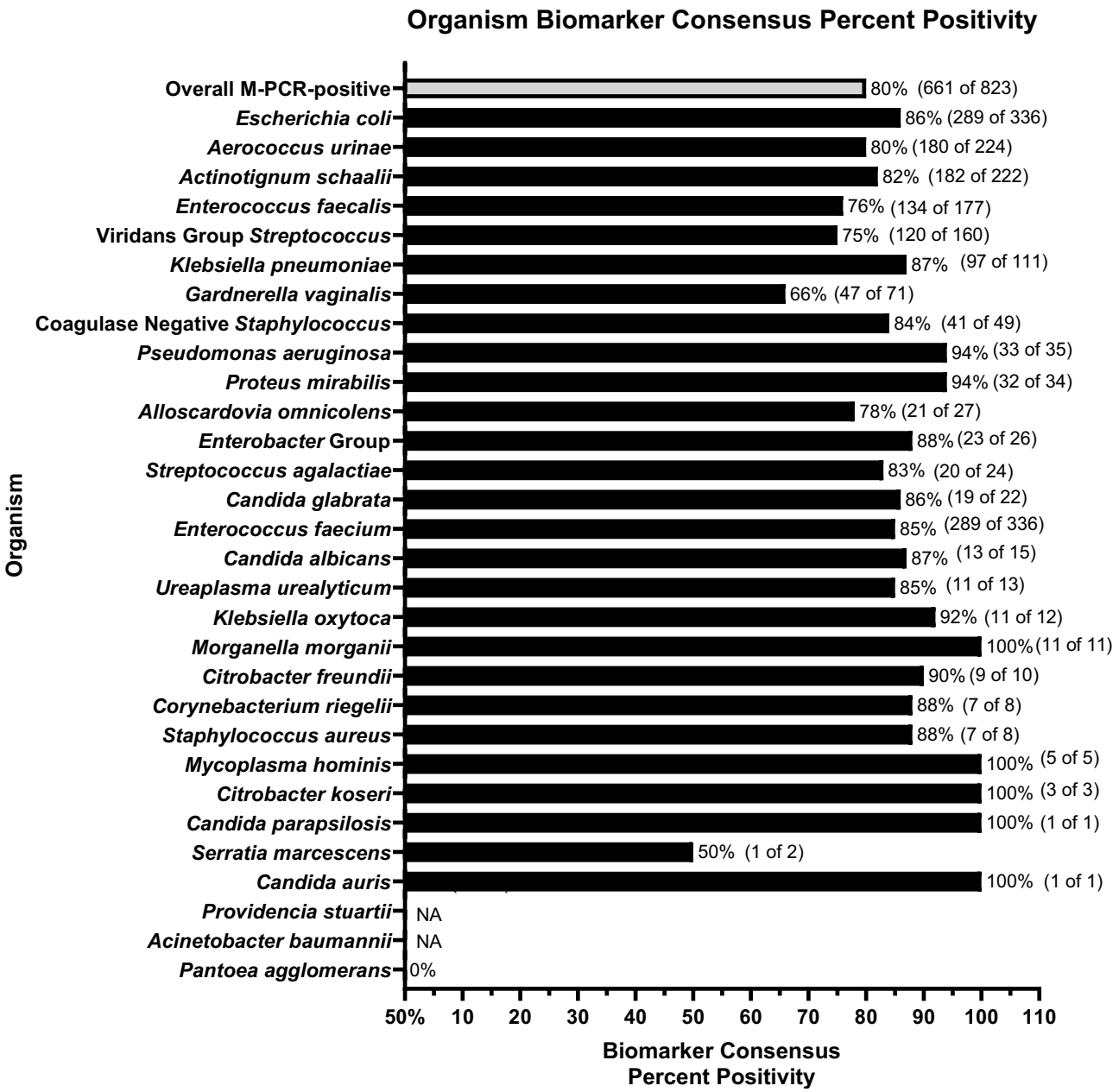

Figure 2 Biomarker consensus percent positivity by microbial identification.
Notes: Organisms or organism groups are listed on the y-axis in descending order of detection prevalence. Bar length along the x-axis represents the percentage of positive specimens (microbial density ≥ 10,000 cells/mL for bacteria/bacterial groups or > 0 cells/mL for yeasts) that are also positive for biomarker consensus. Labels at the end of each bar show the associated biomarker consensus percentage and the number of biomarker consensus positive specimens out of the total number of specimens positive for that organism (in parenthesis).

Using biomarker consensus percent positivity, we then examined whether the detection of a spectrum of organisms present in this assay was associated with positive consensus biomarkers. Overall, 80% of M-PCR-positive specimens were positive for biomarker consensus. Further, of the 28 detected organisms, all but two [*P. stuartii* (n = 1) and *S. marcescens* (n = 2)] had >66% of cases with consensus biomarker positivity. When the organisms were categorized into groups of 5 by decreasing prevalence, all groups showed an association with positive consensus and individual markers. Polymicrobial cases, monomicrobial cases, cases with only classical uropathogens, cases with only emerging uropathogens, cases with *E. coli*, and those without *E. coli* all had elevated biomarkers. These results strongly indicate that the microbes detected by this assay, many of which are fastidious

and emerging pathogens that will likely be missed by SUC, are causative of the UTIs in these cases and not incidental findings. These results make it important to question whether SUC is underdiagnosing due to low sensitivity when the clinical diagnosis from a urology specialty setting, and the urine inflammatory biomarkers agree a UTI is present.

A subset of specimens exhibited outlier data points with low inflammatory biomarker levels despite high microbial densities detected by M-PCR. These outliers with low inflammatory biomarker levels may reflect scenarios where immune responses are compromised due to medications or underlying health conditions, or instances of a resolving UTI.[44–48] They may also be the result of natural variation in the ability of different individual microorganisms and collections of microorganisms to elicit an immune response. Future work could explore such microorganism-specific biomarker thresholds.

Additionally, in a related recent study,[49] both standard urine culture and culture-free M-PCR methods were used to characterize microbial densities of urine specimens from symptomatic presumptive UTI patients for correlation with detected levels of immune response biomarkers NGAL, IL-8, and IL-1β. A significantly higher percentage of SUC-negative specimens were biomarker-positive compared to M-PCR-negative specimens.[49] This suggests that M-PCR has higher sensitivity and specificity for detecting microbes that are causing a UTI, challenging the sensitivity of the current "gold standard" test, SUC, for the identification of uropathogens, and raising questions about false negatives in culture-based testing.

Building upon prior studies,[49,50] this paper shows that symptomatic cases involving non-*E. coli* and emerging uropathogens, along with VGS and CoNS were associated with substantially higher levels of all three biomarkers than in cases where no organisms were detected. These findings suggest that the assay size should not be limited to *E. coli* and a small set of highly prevalent and longstanding uropathogens, which would lead to missed UTI diagnosis and potentially poorer treatment outcomes. Almost all of the organisms present here would be important to include in a UTI assay in order to be confident that most UTI cases could have the causative pathogen identified.

The biggest strength of this study was the comparison of the immune response according to biomarkers NGAL, IL-1β, and IL-8 in a large number of samples obtained from UTI symptomatic patients from a urology specialty setting, against the identification of microorganisms detected by M-PCR from the same urine sample. This approach allowed us to directly associate the presence of microorganisms identified by the M-PCR assay to infection-associated immune responses in the urinary tract of each subject. However, a few organisms in the assay had low detection percentages or were not detected at all, making inferences about their individual clinical relevance uncertain. Future studies with a larger cohort would be helpful to interpreting the individual clinical relevance for these low-prevalence organisms. Additionally, future studies will examine the relationship between these infection-associated urine biomarkers and clinical outcomes data, such as healthcare utilization and the resolution of symptoms upon treatment with antibiotics appropriate for the organism(s) detected by M-PCR.

Conclusions

Measuring microbial detection by M-PCR against positivity rates of three UTI-associated biomarkers (NGAL, IL-8, and IL-1β), we sought to determine whether these 30 microorganisms were clinically relevant for UTI diagnostics. In the study cohort of 1132 individuals ≥60 years of age who were symptomatic for r/cUTI, 28 of 30 microorganisms included in the M-PCR assay were detected. Additionally, 80% of M-PCR positive specimens were positive for biomarker consensus, which in symptomatic patients diagnosed in a specialty setting are a measure of inflammation with high sensitivity and specificity for UTI. Together, these findings demonstrate that the majority of organisms detected by the M-PCR assay are likely clinically relevant with high specificity and that their detection by M-PCR has a low likelihood of false-positivity for UTI.

Disclosure

Drs Natalie Luke and Emery Haley report they are employees of Pathnostics, outside the submitted work; In addition, Dr Natalie Luke has a patent US 10,160,991 issued to Pathnostics, a patent US 11,053,532 issued to Pathnostics, a patent US 17/178,091 pending to Pathnostics, a patent US 17/335,767 pending to Pathnostics, a patent US 17/830,227 pending to Pathnostics, a patent US 18/351,385 pending to Pathnostics, a patent US 18/351,286 pending to Pathnostics, a patent US 63/493,416 pending to Pathnostics, a patent US 63/493,416 pending to Pathnostics, a patent US 63/514,785 issued to Pathnostics, a patent AU 2018254514 B2 issued to Pathnostics, a patent BR112019021943-9 B1 issued to Pathnostics, a patent NZ 759292

issued to Pathnostics; Dr Mohit Mathur reports is an employee of Pathnostics, outside the submitted work; Dr Richard Festa reports is an employee of Pathnostics, during the conduct of the study. Dr David Baunoch reports is an employee and hold stocks from Pathnostics, outside the submitted work; In addition, Dr David Baunoch has a patent US10,160,991 issued to PATHNOSTICS, a patent US11,053,532 issued to PATHNOSTICS, a patent US17/178,091 pending to PATHNOSTICS, a patent US17/335/767 issued to PATHNOSTICS, a patent US17/830/227 pending to PATHNOSTICS, a patent US18/351,286 pending to PATHNOSTICS, a patent PCT/US22/16816 pending to PATHNOSTICS, a patent PCT/US22/77477 pending to PATHNOSTICS, a patent AU2018254514 B2 issued to PATHNOSTICS, a patent BR112019021943-9 B1 issued to PATHONSTICS, a patent NZ759292 pending to PATHNOSTICS, a patent EP3612638 pending to PATHNOSTICS, a patent JP2022-042545 pending to PATHNOSTICS, a patent CA 3,175,879 issued to PATHNOSTICS, a patent CA 3,176,586 issued to PATHNOSTICS, a patent CA 3,061,015 issued to PATHNOSTICS, a patent HK 62020014337.3 issued to PATHNOSTICS, a patent CN 201880039956.9 issued to PATHNOSTICS, a patent IL 294577 issued to PATHNOSTICS. The authors report no other conflicts of interest in this work.

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