Asteroid Publishers

Journal of Surgical Urology

David Baunoch
Research Article
Volume 1 : Issue 1

Received Date : 10 June, 2020
Accepted Date : 09 July, 2020
Published Date : 13 July, 2020

Multisite Prospective Comparison of Multiplex Polymerase Chain Reaction Testing with Urine Culture for Diagnosis of Urinary Tract Infections in Symptomatic Patients

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Citation: Vollstedt A, Baunoch D, Wojno KJ, Luke N, Cline K, et al. (2020) Multisite Prospective Comparison of Multiplex Polymerase Chain Reaction Testing with Urine Culture for Diagnosis of Urinary Tract Infections in Symptomatic Patients. J Sur urology: JSU-102. DOI: 10.29011/ JSU-102.100002

Abstract

Objectives: This study was conducted to compare Multiplex-Polymerase Chain Reaction (M-PCR) with Standard Urine Culture (SUC) for detection and identification of bacteria in Urinary Tract Infection (UTI)-symptomatic patients.

Methods: Both M-PCR and SUC were performed on urine samples from 2511 patients (mean age 73; range 24 - 100) with UTI symptoms.

Results: M-PCR and SUC detected bacteria in 62.7% (1575/2511) and 43.7% (1098/2511) of cases, respectively. SUC detected 21 bacteria, 18 of which were also detected by M-PCR with higher detection rates, especially for the Gram-positive species. M-PCR detected 24 bacteria, among which SUC failed to detect 6, including five Gram-positive bacteria (A. schaalii, A. omnicolens, C. riegelii, M. genitalium, and M. hominis), and one Gram-negative bacterium, (*U. urealyticum*). A total of 590 patients (23.5%) were detected by M-PCR to have at least 1 of the 6 bacteria. A total of 861 polymicrobial infections were reported, with M-PCR reporting 834 (96.9%) and SUC reporting 168 (19.5%). Polymicrobial detections constituted 34.3% (861/2511) of the total patients, 53.0% of M-PCR positives (834/1575) but only 15.3% of SUC positives (167/1098). *A. schaalii*, not detected by SUC, was the most common bacterium [53.0% (442/834)] detected in polymicrobial infections by M-PCR.

Conclusions: This prospective multicenter study of over 2500 UTI-symptomatic patients demonstrated the greater ability of M-PCR to detect bacteria, especially Gram-positive bacteria and polymicrobial infection, over SUC. Use of M-PCR may improve the detection of pathogenic bacteria and consideration of antibiotic resistance and susceptibility may lead to more effective treatment for UTI.

Keywords: Bacteria; Culture; Diagnosis; PCR; Urinary Tract Infection

Abbreviations: AST: Antibiotic Susceptibility Testing; CFU/ml: Colony Forming Units Per Millilitre; CAN: Colistin And Nalidixic Acid Agar; DNA: Deoxyribonucleic Acid; M-PCR: Multiplex Polymerase Chain Reaction; MAC: Macconkey Agar; mL: Millilitre; P-AST: Pooled Antibiotic Susceptibility Testing; PCR: Polymerase Chain Reaction; µL: Microliter; SUC: Standard Urine Culture; UTI: Urinary Tract Infection

Introduction

Urinary Tract Infection (UTI) is the most common infection seen by primary care physicians [1, 2]. The lifetime incidence of UTI in women is 50% to 60%. Recurrent UTI is also common, occurring in 14% to 27% of UTI patients [1, 3, 4]. Initial treatment failure, resulting in recurrent UTI, may occur for various reasons: UTI may be complicated, as in pyelonephritis; patients may not comply with treatment; the diagnosis may be incorrect; and antibiotic resistance may not have been recognized [5, 6]. Accumulating evidence has established that Standard Urine Culture (SUC) may not detect clinically significant bacteria [7]. Reliance on urine culture lead us to believe that urine is sterile. On the other hand, application of molecular methods such as Polymerase Chain Reaction (PCR) have demonstrated that urine is not sterile, even in healthy people [7, 8]. The study described herein was conducted to compare the performance of Multiplex PCR-Based Molecular Testing (M-PCR) with that of SUC for diagnosis of UTI in symptomatic patients.

Methods

Selection and Description of Participants

Study participants were patients who presented with symptoms of UTI at urology clinics. Patients with no symptoms of UTI were not included. Patients were evaluated by any of 75 physicians from 37 urology offices in seven states. A total of 2511 consecutive patients were enrolled between July 26, 2018 and February 27, 2019. All patients provided written informed consent per forms approved by the Western IRB (20181661). Inclusion criteria included patients ≥ 60 years of age presenting with symptoms of acute cystitis, complicated UTI, persistent UTI, recurrent UTIs, prostatitis, and pyelonephritis, and patients at any age presenting with a history and symptoms of interstitial cystitis.

Exclusion criteria included prior participation in this study, antibiotics use for any reason other than UTI at the time of enrollment, presence of chronic (≥ 10 days) indwelling catheters, and self-catheterization. Patients with urinary diversion were excluded from the study. Patients without documented specimen collection and stabilization time, or sufficient specimen volumes to perform both urine culture and the M-PCR test coupled with Pooled Antibiotic Sensitivity Testing (P-AST) were also excluded from the study. Results from the P-AST are discussed in a separate manuscript.

Urine Culture

Urine culture in the study was performed following SUC procedures. Urine samples were obtained from patients by either self-administered clean catch or catheterization. Samples were collected and transported to Pathnostics (Irvine, California) for testing by culture. For culture, urine was vortexed and a sterile plastic loop (1 µL) was used to inoculate blood agar plates. A sterile plastic loop (1 µL) was used also to inoculate Colistin and Nalidixic Acid Agar/Macconkey Agar (CNA/MAC) plates, one loop-full of urine on the CNA side of the plate and another full loop-full on the MAC side of the plate. All plates were incubated at 35º C in 5% CO2 for ≥18 hours and then examined for evidence of growth. Per CLSI Performance Standards for Antimicrobial Susceptibility Testing (30th Edition), plates with < 104 CFU/ml were reported as normal urogenital flora.

For plates with growth (≥ 10^4 CFU/ml), the quantity and morphology of each organism was recorded. The maximum readable colony count using the 1 µL loop is > 10^5 CFU/ml. Colony counts were performed on blood agar plates. Species identification and colony counts were performed on CNA/MAC plates. For plates with ≤ 2 pathogens, species identification and colony counts were reported for each pathogen with ≥ 10^4 CFU/ml. If ≥ 3 pathogens were present, and one or two were predominant, species identification and colony counts were reported. If ≥ 3 pathogens were present without predominant species, a mixed morphotype was reported. Pathogen identification was confirmed with the VITEK 2 Compact System (bioMerieux, Durham, NC) in accordance with standard operating procedures.

Briefly, a sterile swab was used to transfer morphologically similar colonies from positive blood agar plates to prepared polystyrene test tubes containing 3.0 mL of sterile saline. The sample was adjusted for density (equivalent to McFarland No. 0.50 to 0.63). The sample tube and an appropriate identification card were placed into the cassette and inserted into the VITEK 2 instrument. The identity of the bacteria was used to determine Gram status, and a GN card was used for Gram-negative bacteria, and a GP card was used for Gram-positive bacteria. A YST card was used for yeast. Pathogen identification was read from the VITEK 2 instrument.

DNA extraction and M-PCR analysis

DNA was extracted from urine samples with the KingFisher/MagMAX Automated DNA Extraction instrument and the MagMAX DNA Multi-Sample Ultra Kit (ThermoFisher, Carlsbad, CA). Briefly, 400µL of urine were transferred to wells in 96-well deep-well plates, sealed, and centrifuged to concentrate the samples; supernatant was removed. Enzyme Lysis Mix (220µL/well) was added and incubated for 20 min at 65ºC. Proteinase K Mix was added (50µL/well) and incubated for 30 min at 65ºC. Lysis buffer (125 µL/well) and DNA Binding Bead Mix (40 µL/well) were added, and the samples shaken for a minimum of 5 min. The 96-well plate was loaded into the KingFisher/MagMAX Automated DNA Extraction instrument, which was operated in accordance with standard operating procedures.

DNA samples were analyzed with the Pathnostics Guidance® UTI Test. The samples were mixed with universal PCR master mix and amplified with TaqMan technology on a Life Technologies 12K Flex Open Array System. DNA samples were spotted in duplicate on 112-format Open Array chips. Plasmids for each organism being tested for were used as positive controls. Candida tropicalis was used as an inhibition control. A data analysis tool developed by Pathnostics was used to sort data, assess the quality of data, summarize control sample data, identify positive assays, calculate concentrations, and generate draft reports. Probes and primers were used for the following 23 bacteria and 2 bacteria groups:

Bacteria: *Acinetobacter baumannii, Actinotignum schaalii, Aerococcus urinae, Alloscardovia omnicolens, Citrobacter freundii, Citrobacter koseri, Corynebacterium riegelii, Klebsiella aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Mycoplasma genitalium, Mycoplasma hominis, Pantoea agglomerans, Proteus mirabilis, Providencia stuartii, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus agalactiae,* and *Ureaplasma urealyticum.*

Bacterial groups: Coagulase Negative *Staphylococci* Group (CoNS), including *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunesis,* and *Staphylococcus saprophyticus*, and Viridans Group *Streptococci* (VGS), including *Streptococcus anginosus, Streptococcus oralis,* and *Streptococcus pasteuranus.*

The quantities of each of the bacterial species were determined using the standard curve method: first, standard curves of each of the bacterial species were generated from testing replicates of dilution series of known concentrations of the bacterium; constants necessary for the quantitation of each of the bacterial species in unknown samples, such as slope and intercept, were established from each of the standard curves; then PCR Ct values of a target bacterial species from an unknown sample were compared to the standard curve, and the concentration of the target bacterial species (cells/mL) present in the samples was extrapolate and determined. Bacterium with quantity of ≥10,000 cells/mL was defined as "positive" or "detected", and bacteria with quantity < 10,000 cells/mL was defined as "negative" or "not detected".

Statistics

Descriptive statistics were used to describe the data in the study.

Results

Patient Demographics and Clinical Information

Patient demographics and clinical information are given in Table 1. Of the 2511 participants, 1360 (54%) were females and 1151 (46%) were males. The median age in this study was 73 and the range was 24-100 years, with 99% of patients over the age of 60. All patients were presented with symptoms of UTI, such as dysuria, cloudy or strong-smelling urine, pain or pelvic discomfort, fever, and Lower Urinary Tract Symptoms (LUTS). Positive urine analysis, defined as the presence of blood, leukocytes or nitrites, was present in 75.5% of the study population. Antibiotic usages in the last 3 weeks were seen in 16.9% of female and 5.7% of male patients (Table 1).

Variables	All N=2511
Gender	
Female	1360
Male	1151
Age	
<=60, n (%)	25 (1.0)
>60, n (%)	2486 (99.0)
Mean ± SD	73.2 ± 8.4
Min, Max age	24, 100
UTI Symptoms, n (%)	
Dysuria	656 (26.1)
Urine cloudy or strong smell	284 (11.3)
Pain/Pelvic discomfort	588 (23.4)
Fever	42 (1.7)
LUTS	1791 (71.3)
Urinary incontinence	723 (28.8)
Gross hematuria	635 (25.3)
Antibiotic Usage in the Last 3 Weeks, n (%)	291 (11.7)
Positive Urine Analysis or Dipsticks Results, n (%)	1896 (75.5)

Table 1: Patient demographics and clinical information.

M-PCR identified more UTI-symptomatic patients with bacteria in their urine than SUC.

Overall, M-PCR detected bacteria in 62.7% (1575) whereas SUC detected bacteria in 43.7% (1098) of the 2511 patients. SUC and M-PCR were both positive in 1018/2511 (40.5%) cases and were both negative in 856/2511 (34.1%) cases. Only 80 out of the 2511 (3.2%) cases were positive in SUC but negative in M-PCR. On the contrary, 557 out of the 2511 cases (22.2%) were positive in M-PCR but negative in SUC (Table 2). M-PCR detected more bacteria species, especially Gram-positive species, in UTI-symptomatic patients than SUC. The M-PCR panel in this study tested for 23 bacteria plus two bacterial groups. It detected 24 different bacteria or groups in the 2511 patients in the study, and SUC detected 21.

Among the 21 bacteria detected by SUC, 18 were also detected by M-PCR, with higher detection rates by M-PCR than

SUC for almost all of them, especially for the Gram-positive species. For example, *E. faecalis*, CoNS, VGS, and *A. urinae*, were detected in 200 (8%), 72 (2.9%), 49 (2%), and 21 (0.8%) in the 2511 patients by SUC, and were 253 (10.1%), 309 (12/3%), 439 (17.5%), and 460 (18.3%) by M-PCR (Figure 1). *E. coli* was the most detected bacterium by both SUC and M-PCR, with detection rates of 21.2% and 22.7%, respectively. The top bacterial species detected by SUC, defined as at least a 1% detection rate) included 8 bacterial species, where M-PCR detected 13 bacteria with at least a 1% detection rate, including all 8 that were detected by SUC (Figure 1).

Among the 24 bacteria detected by M-PCR, SUC failed to detect 6 of them, including five Gram-positive bacteria (*A. schaalii*, *A. omnicolens*, *C. riegelii*, *M. genitalium*, and *M. hominis*), and one Gram-negative bacterium, *U. urealyticum*. Four of the 6 were on the top list detected by M-PCR (Figure 1). A total of 590 patients (23.5%) were detected by M-PCR to have at least 1 of the 6 bacteria species in their urine samples. There were 3 bacterial species detected by SUC that were not detected by the M-PCR. They were the *Enterobacter* species, the *Enterococcus* species, and the other species, detected in only 0.9%, 0.2% and 0.9%, of all patients, respectively. This is not due to the failure of the M-PCR methods, but rather that the M-PCR panel did not include primers and probes for the three bacteria (Figure 1). Bacteria detected by SUC or M-PCR in male and female patients were detailed in (Supplementary Table 1).

	M-PCR Positive	M-PCR Negative	Total
SUC Positive	1018 (40.5%)	80 (3.2%)	1098 (43.7%)
SUC Negative	557 (22.2%)	856 (34.1%)	1413 (56.3%)
Total	1575 (62.7%)	936 (37.3%)	2511 (100%)

Table 2: Agreement of M-PCR and SUC in patients with UTI symptoms.

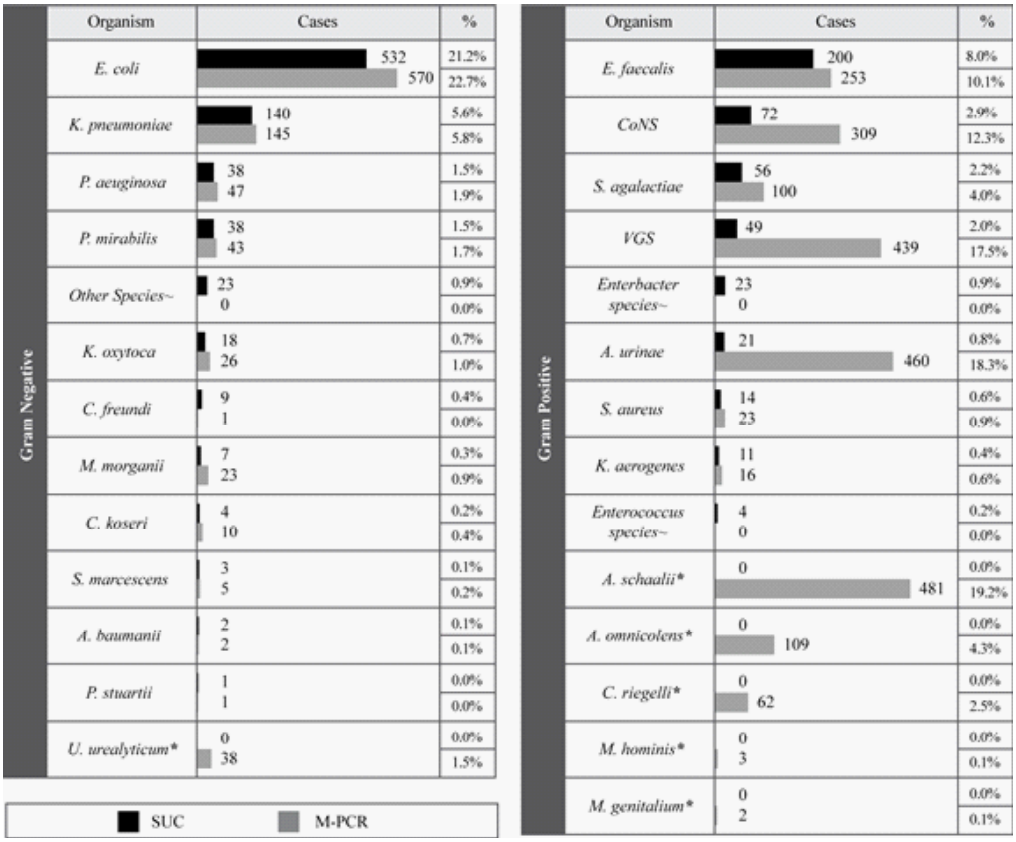

Figure 1: Number of patients with UTI symptoms tested positive for various bacteria by SUC or M-PCR, listed in descending order according to SUC results, Gram-negative organisms grouped on the left and Gram-positive organisms grouped on the right. Among the 24 bacteria detected by M-PCR, SUC failed to detect 6 of them (marked with *), including five Gram-positive bacteria. There were 3 bacterial species (marked with ~), detected by SUC that were not detected by the M-PCR because the panel did not include primers and probes for those three bacteria. Abbreviations: *CoNS,* Coagulase Negative *Staphylococci; VGS,* Viridans Group *Streptococci.*

**: Bacteria or bacterial species only detected by M-PCR.*

~: Bacterial species only detected by SUC.

Organisms	SUC N=2511		M-PCR N=2511	
	Female N=1360	Male N=1151	Female N=1360	Male N=1151
	n (%)	n (%)	**n (%)**	**n (%)**
A. baumannii	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)
A. omnicolens	/	/	98 (7.2)	11 (1)
A. schaalii	/	/	372 (27.4)	109 (9.5)
A. urinae	17 (1.3)	4 (0.3)	340 (25)	120 (10.4)
C. freundii	8 (0.6)	1 (0.1)	1 (0.1)	0 (0)
C. koseri	2 (0.1)	2 (0.2)	3 (0.2)	7 (0.6)
C. riegelli	/	/	56 (4.1)	6 (0.5)
CoNS	24 (1.8)	48 (4.2)	178 (13.1)	131 (11.4)
K. aerogenes	7 (0.5)	4 (0.3)	12 (0.9)	4 (0.3)
E. coli	420 (30.9)	112 (9.7)	456 (33.5)	114 (9.9)
E. faecalis	121 (8.9)	79 (6.9)	155 (11.4)	98 (8.5)
Enterobacter species	10 (0.7)	13 (1.1)	/	/
Enterococcus species	3 (0.2)	1 (0.1)	/	/
K. oxytoca	8 (0.6)	10 (0.9)	15 (1.1)	11 (1)
K. pneumoniae	100 (7.4)	40 (3.5)	107 (7.9)	38 (3.3)
M. genitalium	/	/	0 (0)	2 (0.2)
M. hominis	/	/	2 (0.1)	1 (0.1)
M. morganii	2 (0.1)	5 (0.4)	13 (1)	10 (0.9)
P. aeruginosa	22 (1.6)	16 (1.4)	25 (1.8)	22 (1.9)
P. mirabilis	21 (1.5)	17 (1.5)	25 (1.8)	18 (1.6)
P. stuartii	0 (0)	1 (0.1)	0 (0)	1 (0.1)
Other species	42 (3.1)	14 (1.2)	/	/
S. agalactiae	7 (0.5)	7 (0.6)	69 (5.1)	31 (2.7)
S. aureus	1 (0.1)	2 (0.2)	14 (1)	9 (0.8)
S. marcescens	40 (2.9)	9 (0.8)	2 (0.1)	3 (0.3)
U. urealyticum	/	/	29 (2.1)	9 (0.8)
VGS	15 (1.1)	8 (0.7)	385 (28.3)	54 (4.7)

Supplementary Table 1: Bacteria detected by SUC or M-PCR in male and female patients with UTI symptoms. *P. agglomerans* was not listed in the table since it was not detected among the 2511 patients. Abbreviations: *CoNS,* Coagulase Negative *Staphylococci; VGS,* Viridans Group *Streptococci.*

M-PCR detected more polymicrobial infections in UTI-symptomatic patients than SUC.

In this study, SUC was found to have limited capacity to detect polymicrobial infections compared with M-PCR (Table 3). The specific bacteria identified in polymicrobial infections are listed in Figure 2. Eight hundred sixty-one polymicrobial infections were detected by M-PCR and SUC combined. Among these detections, M-PCR detected 834 of 860 (96.9%), SUC detected 168 of 860 (19.5%) and 141 of 860 (16.4%) were polymicrobial by both M-PCR and SUC. Polymicrobial infections were detected in 34.3% (861/2511) of patients, 53.0% of M-PCR positives (834/1575) but only 15.3% of SUC positives (167/1098) (Table 3).

One of the six bacteria that were detected by M-PCR but not by SUC, *A. schaalii*, was the most common bacterium involved in polymicrobial infections and was involved in 53.0% (442/834) of all polymicrobial detections by M-PCR. The total cases detected with *E. coli* were similar between SUC and M-PCR. However, SUC reported that *E. coli* was most commonly a monomicrobial infection where M-PCR reported *E. coli* most commonly as a polymicrobial infection. Similar results were observed in other bacteria species, such as *E. faecalis*, *K. pneumoniae*, and CoNS (Figure 2) (Supplementary Table 2).

		M-PCR			
		Polymicrobial	Monomicrobial	Negative	Total
SUC	Polymicrobial	141 (5.6%)	24 (1.0%)	2 (0.1%)	167 (6.7%)
	Monomicrobial	425 (16.9%)	428 (17.1%)	78 (3.1%)	931 (37.1%)
	Negative	268 (10.7%)	289 (11.5%)	856 (34.1%)	1413 (56.3%)
	Total	834 (33.2%)	741 (29.5%)	936 (37.3%)	2511 (100.0%)

Table 3: Agreement of M-PCR and SUC in detecting monomicrobial and polymicrobial infections in patients with UTI symptoms.

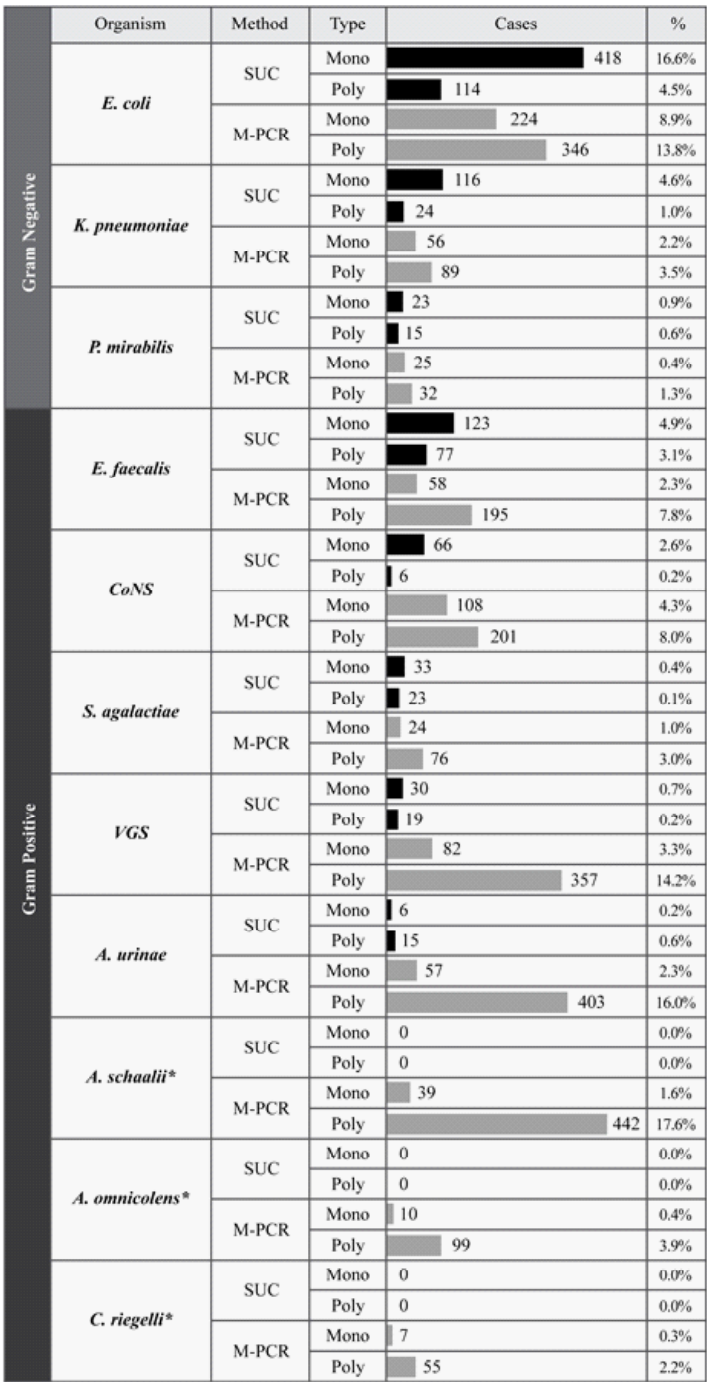

Figure 2: Bacteria detected in polymicrobial infections by SUC and M-PCR. **Abbreviations:** Abbreviations: Mono: Monomicrobial; Poly: Polymicrobial; CoNS: Coagulase Negative *Staphylococci*; VGS: Viridans Group *Streptococci*.

**: Bacteria or bacterial species only detected by M-PCR.*

Organism	SUC N=2511		M-PCR N=2511	
	Monomicrobial	Polymicrobial	Monomicrobial	Polymicrobial
	n (%)	n (%)	n (%)	n (%)
A. baumannii	1 (0)	1 (0)	0 (0)	2 (0.1)
A. omnicolens	/	/	10 (0.4)	99 (3.9)
A. schaalii	/	/	39 (1.6)	442 (17.6)
A. urinae	6 (0.2)	15 (0.6)	57 (2.3)	403 (16)
C. freundii	8 (0.3)	1 (0)	0 (0)	1 (0)
C. koseri	3 (0.1)	1 (0)	2 (0.1)	8 (0.3)
C. riegelii	/	/	7 (0.3)	55 (2.2)
CoNS	66 (2.6)	6 (0.2)	108 (4.3)	201 (8)
K. aerogenes	8 (0.3)	3 (0.1)	4 (0.2)	12 (0.5)
E. coli	418 (16.6)	114 (4.5)	224 (8.9)	346 (13.8)
E. faecalis	123 (4.9)	77 (3.1)	58 (2.3)	195 (7.8)
Enterobacter species	18 (0.7)	5 (0.2)	/	/
Enterococcus species	3 (0.1)	1 (0)	/	/
K. oxytoca	13 (0.5)	5 (0.2)	6 (0.2)	20 (0.8)
K. pneumoniae	116 (4.6)	24 (1)	56 (2.2)	89 (3.5)
M. genitalium	/	/	2 (0.1)	0 (0)
M. hominis	/	/	0 (0)	3 (0.1)
M. morganii	6 (0.2)	1 (0)	5 (0.2)	18 (0.7)
P. aeruginosa	25 (1)	13 (0.5)	24 (1)	23 (0.9)
P. mirabilis	23 (0.9)	15 (0.6)	11 (0.4)	32 (1.3)
P. stuartii	0 (0)	1 (0)	0 (0)	1 (0)
Other species	33 (1.3)	23 (0.9)	/	/
S. agalactiae	11 (0.4)	3 (0.1)	24 (1)	76 (3)
S. aureus	2 (0.1)	1 (0)	11 (0.4)	12 (0.5)
S. marcescens	30 (1.2)	19 (0.8)	2 (0.1)	3 (0.1)
U. urealyticum	/	/	9 (0.4)	29 (1.2)
VGS	18 (0.7)	5 (0.2)	82 (3.3)	357 (14.2)

Supplementary Table 2: Monomicrobial and polymicrobial bacteria detected by SUC or M-PCR. *P. agglomerans* was not listed in the table since it was not detected among the 2511 patients. Abbreviations: CoNS, Coagulase Negative *Staphylococci*; VGS, Viridans Group *Streptococci*.

Discussion

This prospective study of over 2500 patients with symptoms of UTI found that M-PCR was positive for bacteria in 62.7% of patients versus 43.7% with SUC. Twenty-two percent of PCR-positive patients had a negative urine culture and PCR detected

five times more polymicrobial infections than did urine culture (834 vs. 167). These results are consistent with previous studies that have also shown that PCR provides advantages in detection sensitivity and identification specificity for the diagnosis of UTI [9-11]. PCR is expanding our understanding of the urobiome and alterations with infection and disease. SUC has a limited ability of to grow fastidious organisms, especially Gram-positive bacteria. As a result, PCR reports more bacteria, more different bacteria and more bacterial combinations. Others have demonstrated the poor performance of SUC.

Hilt showed that in symptomatic and asymptomatic patients 92% had negative SUC, yet 80% of these were positive when using expanded quantitative urine culture techniques to improve growth of fastidious organisms [8]. Khasriya showed that culture of the shed urothelial cells (where uropathogens adhere) resulted in large numbers or bacteria that were undetected on SUC [12]. Kline reported that Gram-positive bacteria including *Aerococcus, Actinobaculum* and *Corynebacterium*, have been overlooked as uropathogens because of the limitations of the SUC [13]. In this study, six bacteria detected by M-PCR were not detected by culture and five of them were Gram-positive. Three of these bacteria (*A. schaalii, A. omnicolens*, and *C. riegelii*) were among the 10 most frequently detected bacteria by M-PCR, combining for 22.3% of all bacterial detections.

Even more, *A. schaalii* was found in polymicrobial infections more often than any other bacterium: it was involved in 53.0% (442/834) of all M-PCR detected polymicrobial infections. Five of the six have also been reported as pathogenic for UTI and associated with increased risk for sepsis and bacteremia, endocarditis, Fournier's gangrene, and abdominal abscess [14-16]. Of the others, *A. schaalii* and *A. omnicolens* are acknowledged as uropathogens [17, 18]. *C. riegelii* is commonly regarded as a commensal but has also been shown to cause UTI [19, 20]. In addition, both *M. hominis* and *U. urealyticum* have been documented as causing LUTS, and may be antibiotic resistant [21, 22]. Therefore, one cannot say that the organisms identified by PCR are not pathogenic, instead, that we do not yet understand their role in the normal urobiome or in a urinary tract infection.

Studies like these are among the first to investigate these patients with both SUC and PCR and further work is ongoing. The M-PCR method detected five times as many polymicrobial UTI's than traditional culture. SUC is inherently limited in its ability to detect and identify pathogens in polymicrobial infections [23, 24]. The proportion of the total number of patients with M-PCR detected polymicrobial infections in this study was 33.2%, which is consistent with previous reports [7, 8]. Importantly, among patients who tested positive for bacteria with M-PCR, there were more polymicrobial infections (53.0%, 834/1575) than monomicrobial infections (47.0%, 741/1575). The study of urine bacteria by PCR in the patient with UTI symptoms will help us identify the abnormalities of the urobiome.

The large number of Gram-positive organisms in the urine identified in this and other studies may indicate an important role in the normal urobiome and in the pathogenesis of polymicrobial UTI, perhaps a critical role in the development of consortia, non-random communities of microbes that interact synergistically in providing community members with growth and survival advantages. Failure of standard culture to detect and identify bacteria also raises the possibility of treatment failure due to unidentified antibiotic resistance. Several papers have reported that clinical antibiotic resistance, i.e., antibiotic response in the patient, was different from resistance predicted on the basis of results of laboratory tests [25, 26]. This difference could occur for a number of reasons, but failure to grow bacteria will lead to failures of sensitivity testing.

Our data showed that the M-PCR/P-AST test takes an average of 29.7 hours (9 hours less than SUC) to provide physicians with urine pathogen and drug sensitivity results. This difference is increase to a median of 19 hours (34.5 hours and 53.7 hours, for M-PCR/P-AST and SUC, respectively) for patients for both positive pathogen identification and susceptibility results. The faster turnaround time also places M-PCR in an advantageous position, compared to SUC.

The current study was not designed to test the clinical advantage of M-PCR over SUC. All bacteria on the PCR panel and all bacteria detected in SUC have previously been described as causing UTI [7, 8]. However, all bacteria do not contribute equally to pathogenesis. This is especially true for polymicrobial infections. Some bacteria may be more pathogenic than others, and interactions among bacteria in polymicrobial infections have yet to be described.

The identification of more and different bacteria in urine from patients with UTI by PCR does not prove the bacteria are the cause of the patients' symptoms. Proof of this relationship will depend on documenting that eradication of the putative pathogens results in resolution or improvement of symptoms. The detection criterion of ≥10,000 cells/mL for M-PCR was also noteworthy.

The threshold of ≥10,000 cells/mL was selected to be consistent with the current standard of care. Studies have shown that colony counts as low as 10^2 bacteria/mL are clinically significant [27]. Others have argued that reducing the threshold will ensure physicians do not miss true cases of UTI's and that patients will be treated properly [28, 29]. The caveat is that patients must be symptomatic of a UTI as it is demonstrated that the bladder contains a microbiome [7, 8, 27, 29].

In addition to bacteria, we examined the fungus and virus detection in symptomatic patient urine samples. There were several cases for which yeast and viral particles were identified in combination with bacteria. Likewise, there were cases for which the fungus and virus were found independent of bacteria. These microbes may also be pathogenic. While this paper focuses on comparing bacteria in culture versus M-PCR, other articles will focus on fungal and virus detection.

Strengths of this study include the large number of

symptomatic patients recruited from 37 different urology clinics in seven states, all serving in a community setting. The patients participating in the study likely reflect the larger population of UTI patients, to evaluate the performance of the increased sensitivity and specificity of PCR. These results should be relevant to clinicians attending to patients who present with UTI symptoms. The results of this prospective study of over 2500 patients with symptoms of urinary tract infection demonstrated the greater ability of M-PCR to detect bacteria over SUC, especially for detection of Gram-positive organisms and polymicrobial infections. Use of M-PCR for evaluation of urinary tract infections might improve detection of pathogenic bacteria and consideration of antibiotic resistance and susceptibility. These advantages, in turn, might lead to more effective treatment [30-33].

Acknowledgements

All funding was provided by Pathnostics and Thermo Fisher.

Conflicts of Interest

David Baunoch and Natalie Luke are employees of Pathnostics, Inc. Megan Campbell was an employee of Pathnostics, Inc at the time of the study.

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