Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients

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OBJECTIVE To evaluate whether multiplex PCR-based molecular testing is noninferior to urine culture for detection of bacterial infections in symptomatic patients.

METHODS Retrospective record review of 582 consecutive elderly patients presenting with symptoms of lower urinary tract infection (UTI) was conducted. All patients had traditional urine cultures and PCR molecular testing run in parallel.

RESULTS A total of 582 patients (mean age 77; range 60-95) with symptoms of lower UTI had both urine cultures and diagnostic PCR between March and July 2018. PCR detected uropathogens in 326 patients (56%, 326/582), while urine culture detected pathogens in 217 patients (37%, 217/582). PCR and culture agreed in 74% of cases (431/582): both were positive in 34% of cases (196/582) and both were negative in 40% of cases (235/582). However, PCR and culture disagreed in 26% of cases (151/582): PCR was positive while culture was negative in 22% of cases (130/582), and culture was positive while PCR was negative in 4% of cases (21/582). Polymicrobial infections were reported in 175 patients (30%, 175/582), with PCR reporting 166 and culture reporting 39. Further, polymicrobial infections were identified in 67 patients (12%, 67/582) in which culture results were negative. Agreement between PCR and urine culture for positive cultures was 90%, exceeding the noninferiority threshold of 85% (95% conflict of interest 85.7%-93.6%).

CONCLUSION Multiplex PCR is noninferior to urine culture for detection and identification of bacteria. Further investigation may show that the accuracy and speed of PCR to diagnose UTI can significantly improve patient outcomes. UROLOGY 136: 119–126, 2020. © 2019 Elsevier Inc.

Traditional urine culture is commonly regarded as the gold standard for detection and identification of pathogens. However, evidence has been accumulating to support use of molecular methods such as PCR. With antimicrobial resistance becoming both more common and complex, effective treatment of (urinary tract infection) UTIs is even more dependent on the accurate identification of pathogens. Some organisms can be fastidious, and therefore difficult to grow in culture. Further, PCR results can be obtained in a day or less, while culture can require 2 or more days. Previous studies have reported PCR to have both superior sensitivity and specificity, and have recommended PCR for rapid identification of pathogens in sepsis,[1-3] and for diagnosis of genital infections and sexually transmitted diseases,[4-6] parasitic infections,[7] tuberculosis,[8] and gastrointestinal infections.[9]

Few studies have compared multiplex PCR with urine culture for diagnosis of UTIs and acute cystitis. Although several studies have compared performance of PCR with urine culture for detection of a single pathogen, only 4 have tested multiplex PCR: one against 15 bacteria,[10] a second against 14 bacteria together with 6 fungi,[11] a third against 20,[12] and the fourth against 9 bacteria.[13] Polymicrobial infections may occur in as many of 39% of UTIs[14,15] and can display enhanced virulence and

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increased antibiotic resistance.[16] Simultaneous detection of a larger number of pathogens may confer benefits for outcome of UTIs. The long-term objective is to determine whether the speed and accuracy of multiplex PCR improves patient care and potentially reduces costs. This study is the first step to confirm that PCR is noninferior to traditional urine culture in detecting bacteria in symptomatic patients. In this study, we compared a multiplex panel of 31 bacteria against urine culture for diagnosis of patients presenting with symptoms of UTI. Though this PCR also reports resistance for each organism, these data are not reported here. Although it may sound intuitive that the resistance of a single organism is different from the susceptibility results of the polymicrobial "soup", the data are being analyzed to better understand the patterns of the results.

MATERIALS AND METHODS

Participants

This was a single-site (Comprehensive Urology, Royal Oak, MI) retrospective study. IRB approval was obtained prior to commencing the study (IRB protocol number: 20171870). All patients meeting the inclusion criteria presenting to clinic between March and July 2018 (n = 582) were included in the study. Inclusion criteria: ≥60 years of age; symptoms of acute cystitis or UTI; sufficient urine sample volume for urinalysis, traditional culture, and PCR; all samples were shipped FedEx priority overnight. All samples except 4 were received by the laboratory the day after collecting the samples. Four samples were received 2 days after collection.

This study focused on patients ≥60 years of age. UTI is common in this age group and can be more difficult to diagnose. Localized urogenital symptoms may not be present in this population and differentiation between UTI and asymptomatic bacteriuria can be difficult.[17] Patients in this age group could benefit significantly from better identification of UTI pathogens.

Urine Culture

Study participants provided a urine sample obtained either by self-administered clean catch or by catheterization. The urine was mixed and a sterile plastic loop (1 μL) used to inoculate blood agar plates. A sterile plastic loop (1 μL) was also used 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 35C in 5% CO_2 for ≥18 hours and then examined for evidence of growth. Plates with $<10^4$ CFU/mL were reported as normal urogenital flora. For plates with growth ($\geq 10^4$ 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 the 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 $\geq 10^4$ CFU/mL. If ≥3 pathogens were present, and one or 2 were predominant, species identification, and colony counts were reported. If ≥3 pathogens were present without predominant species, a mixed morphotype was reported.

Samples for Gram stain were prepared by applying a thin, even smear on microscope slides, allowed to air dry, and then fixed with methanol. The slides were covered in crystal violet solution for one minute, rinsed with water, covered with iodine for 1 minute, and then rinsed with water again. *Staphylococcus aureus* 29213 was used as a positive control, and *E. coli* 35218 was used as a negative control.

Pathogen identification was conducted using 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. A GN card was used for Gram negative bacteria, and a GP card used for Gram positive bacteria. An YST card was used for yeast. Pathogen identification was then read from the VITEK 2 instrument.

DNA Extraction and Analysis

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

DNA samples were analyzed using the Pathnostics Guidance UTI Test. Samples were mixed with universal PCR master mix and amplified using TaqMan technology on a Life Technologies 12K Flex OpenArray System. DNA samples were spotted in duplicate on 112-format OpenArray 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 pathogens:

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

Bacterial Groups: Coagulase negative staphylococci (*Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunesis*, *Staphylococcus saprophyticus*); Viridans group streptococci (*Streptococcus anginosus*, *Streptococcus oralis*, *Streptococcus pasteuranus*)

STATISTICAL ANALYSIS

Demographics and symptoms were compared for male and female patients with 2-sample *t* tests or Fisher's exact tests,

as appropriate. Noninferiority of Guidance UTI to traditional culture in terms of detecting bacterial infections was assessed by comparing the lower 95% Wilson-score confidence interval (CI) for the positive percentage agreement to the noninferiority threshold of 85%. Incidences of bacterial infections in males and females (according to PCR and culture) were compared with Fisher's exact tests.

The required sample size to yield 90% power to conclude noninferiority of Guidance UTI relative to traditional urine C&S was calculated assuming a Guidance UTI sensitivity (relative to culture) of 91% and assuming 60% of patients would test positive by culture (based on results in PGX-031 UTI TaqMan QuantStudio 12K Flex Data Analysis). This sample size was calculated in the Tests for One Proportion Procedure of NCSS Power Analysis Statistical Software, Version 14. A sample size of 310 patients with positive culture tests yields 90% power to conclude noninferiority. Assuming 60% of patients test positive for culture, a total of 517 patients should be enrolled in the study.

RESULTS

A total of 582 patients, mean age 77 (range 60-95) with symptoms of lower UTI had both urine culture and diagnostic PCR between March and July 2018 (Table 1). Sixty percent (60%, 347/582) were male and 40% (235/582) were female. Clinical symptoms included dysuria (38%, 221/582), incontinence (33%, 192/582), urine that was cloudy or had an odor (23%, 133/582), and pain or discomfort (7%, 40/582).

Sixty percent of patients (60%, 347/582) had positive results by PCR, urine culture, or both. PCR detected bacteria in 56% of patients (326/582), while urine culture detected pathogens in 37% of patients (217/582) (Table 2). PCR and culture agreed in 74% of cases (431/582): both PCR and culture were positive in 34% of patients (196/582), and both were negative in 40% (235/582). There was disagreement between PCR and culture in 26% of cases (151/582): PCR was positive while culture was negative in 22% of patients (130/582), and PCR was negative while culture was positive in 4% (21/582). The agreement between PCR and urine culture for positive cultures was 196/217 (90%), exceeding the noninferiority threshold of 85% (95% CI: 85.7%-93.6%).

The multiplex panel used in this study tested for 31 bacteria, and PCR and urine culture together identified 29 different bacterial pathogens. PCR detected 24 bacteria, while culture detected 21 different bacteria.

The most common organisms detected by PCR were *E. coli* (29% of PCR positives, 93/326), *Actinobaculum schaali* (27% of PCR positives, 89/326) and *Viridans group Streptococci* (27% of PCR positives, 89/326) (Fig. 1). Traditional culture failed to detect *Actinobaculum schaali* (n = 0), and *Viridans group Streptococci* was isolated in culture only rarely (6% of culture positives, 14/217). *E. coli* was also the most common bacterium detected by culture (34% of culture positives, 74/217) and *Enterococcus faecalis* was the second most common (21% of culture positives, 46/217).

There were 8 bacteria that were identified only by PCR and 5 bacteria that were only detected by culture (Table 3). These 5 bacteria identified only by culture were not included on the multiplex PCR panel. These 5 bacteria were only detected in 8 patients by culture, accounting for 4% of the total positive

Table 1. Study participant demographics and symptoms

Parameter	*Total*	*Male*	*Female*	*p Value**
Age (years)				
n	582	347	235	
Mean (SD)	77.2 (7.9)	76.9 (7.9)	77.7 (7.9)	.01
Median	77	76	79	
Min, Max	60, 95	60, 95	60, 95	
Symptoms — n (%)				
Dysuria	219 (37.6%)	110 (31.7%)	109 (46.4%)	.0005
Urinary incontinence	196 (33.7%)	145 (41.8%)	51 (21.7%)	<.0001
Cloudy or Strong-smelling urine	132 (22.7%)	75 (21.6%)	57 (24.3%)	.48
Pain	18 (3.09%)	7 (2.02%)	11 (4.68%)	.09
Abdominal	7 (1.20%)	3 (0.86%)	4 (1.70%)	.45
Flank	5 (0.86%)	1 (0.29%)	4 (1.70%)	.16
Lower back	4 (0.69%)	1 (0.29%)	3 (1.28%)	.31
Penis/scrotum	2 (0.34%)	2 (0.58%)	0 (0.00%)	1.00
Pelvic discomfort	24 (4.12%)	6 (1.73%)	18 (7.66%)	.001
Lower grade fever	1 (0.17%)	1 (0.29%)	0 (0.00%)	1.00
Agitation	2 (0.34%)	2 (0.58%)	0 (0.00%)	.52
Frequency	10 (1.72%)	4 (1.15%)	6 (2.55%)	.21
Nocturia	1 (0.17%)	0 (0.00%)	1 (0.43%)	.40
Patient-reported hematuria	1 (0.17%)	0 (0.00%)	1 (0.43%)	.40
Dipstick results — n (%)				
Hematuria	246 (42.7%)	138 (40.4%)	108 (46.2%)	.17
Atypical symptoms — n (%)				
Increased falls/Tripping/Tired/ Feeling Ill/Decline in ADLs	7(1.2%)	6 (1.73%)	0 (0.00%)	1.00
Antibiotic usage — n (%)				
Antibiotic treatment in last 3 weeks	89 (15.3%)	30 (8.65%)	59 (25.1%)	<.0001

* p values are from Fisher's exact test or a t-test, as appropriate.

Table 2. Agreement of PCR and traditional urine culture in patients with clinical symptoms of UTI

			PCR					
			Positive			Negative	Total	Agreement
			Polymicrobial	Monomicrobial	Total Positive			
Culture	Positive	Polymicrobial	30 (5.2%)	3 (0.5%)	33 (5.7%)	6 (1.0%)	39 (6.7%)	30/39 (76.9%)
		Monomicrobial	69 (11.9%)	94 (16.2%)	163 (28.0%)	15 (2.6%)	178 (30.6%)	94/178 (52.8%)
		Total Positive	99 (17.0%)	97 (16.7%)	196 (33.7%)	21 (3.6%)	217 (37.3%)	196/217 (90.3%)
	Negative		63 (10.8%)	130 (22.3%)	235 (40.4%)	365 (62.7%)	235/365 (64.4%)	
	Total		166 (28.5%)	326 (56.0%)	256 (44.0%)	582	(196+235)/582 (74.1%)	
	Agreement		30/166 (18.1%)	94/160 (58.8%)	196/326 (60.1%)	235/256 (91.8%)	(196+235)/582 (74.1%)	

The agreement between PCR and urine culture for positive cultures was 196/217 (90%), exceeding the noninferiority threshold. Polymicrobial infections are those having ≥2 organisms infections. PCR was significantly more sensitive than urine culture for detecting polymicrobial infections.
Polymicrobial percentage agreement (%, 95% CI) 76.9% (61.7, 87.4%).
Monomicrobial percentage agreement (%, 95% CI) 52.8% (45.5, 60.0%).
Negative percentage agreement (%, 95% CI) 64.4% (59.3, 69.1%).
Overall percentage agreement (%, 95% CI) 61.7% (57.7, 65.5%).

culture samples (8/217): *Enterobacter cloacae* as monomicrobial infection in 2 patients (1%, 2/217), *Enterococcus faecium* alone in 1 patient (0.5%, 1/217), *Enterobacter cloacae* and *faecium* in 1 patient (0.5%, 1/217), *Enterobacter cloacae* and *E. coli* in 1 patient (0.5%, 1/217), *Proteus mirabilis* and *Streptococcus gallolyticus* in 1 patient (0.5%, 1/217), and *Kocuria rosea* and *Kocuria kristinae* in 1 patient each (0.5%, 1/217), (Table 3). Three of these 8 patients tested positive to other bacteria by PCR, 1 tested positive to *Candida albicans*, and 1 tested positive to JC virus by PCR. By contrast, there were 108 patients who tested positive for at least one of the 8 bacteria that were detected by PCR but not by culture. Only 45 of the 108 (42%) tested positive to other bacteria by culture.

In 88 patients (15%, 88/582), both PCR and urine culture identified the same pathogens and the same number of bacteria. In 85/88 cases (97%) both culture and PCR reported a single pathogen. The most common bacteria with total agreement were *E. coli* (35%, 30/85), *Enterococcus faecalis* (19%, 16/85), and *Klebsiella pneumoniae* (14%, 12/85). Together, these 3 bacteria accounted for 58 of 85 cases (68%) of single bacteria total agreement, but only 29% (194/661) of the total bacteria reported by PCR.

There were 175 cases of polymicrobial infection, defined as ≥2 bacteria. PCR detected 166 and culture detected 39 (Table 2). There were 30 cases in which both PCR and culture reported polymicrobial infections. There were 3 cases in which culture reported a polymicrobial infection while PCR reported a monomicrobial infection, and an additional 6 cases in which culture reported a polymicrobial infection and PCR was negative. In 1 of these 6 cases, both bacteria detected by culture were not included on the PCR panel; in another case, one of the bacteria detected by culture was not included on the PCR panel. Although it occurs rarely, molecular inhibition can cause PCR tests to be negative. This may have happened with the other 4 cases, which amount to less than 1% (4/582) of patients tested.

In 67 polymicrobial cases (12% of patients, 67/582; 38% of polymicrobial infections, 67/175) culture was negative. Of the 30 cases in which PCR and culture detected polymicrobial infections, 7 were labelled as "Mixed" by culture so there could be no agreement on the organisms detected, 23 had at least one of the culture detected organisms detected by PCR and 17 had 2 of the culture detected organisms detected by PCR. There were 3 cases in which 2 pathogens were reported and there was total agreement between PCR and culture. In 2 cases, the pathogens were *E. coli* and *Enterococcus faecalis;* in 1 case, the pathogens were *Proteus mirabilis* and *Enterococcus faecalis*. Although there were 92 cases of at least 3 pathogens, there were none in which PCR and culture agreed on the organisms detected, since culture did not identify the organisms in most of these cases, but just labelled them as "Mixed". Which bacteria cause infection and inflammation in polymicrobial situations is impossible to determine.

DISCUSSION

This study showed that multiplex PCR is not inferior to traditional urine culture, and in fact detected bacteria in 36% of symptomatic patients who had a negative urine culture. In addition, multiplex PCR detected more polymicrobial infections than urine culture, 28% of patients, compared to 7% of patients. In addition to higher detection rates, PCR can provide results in as little as 6 hours compared to traditional culture which takes 48 or more

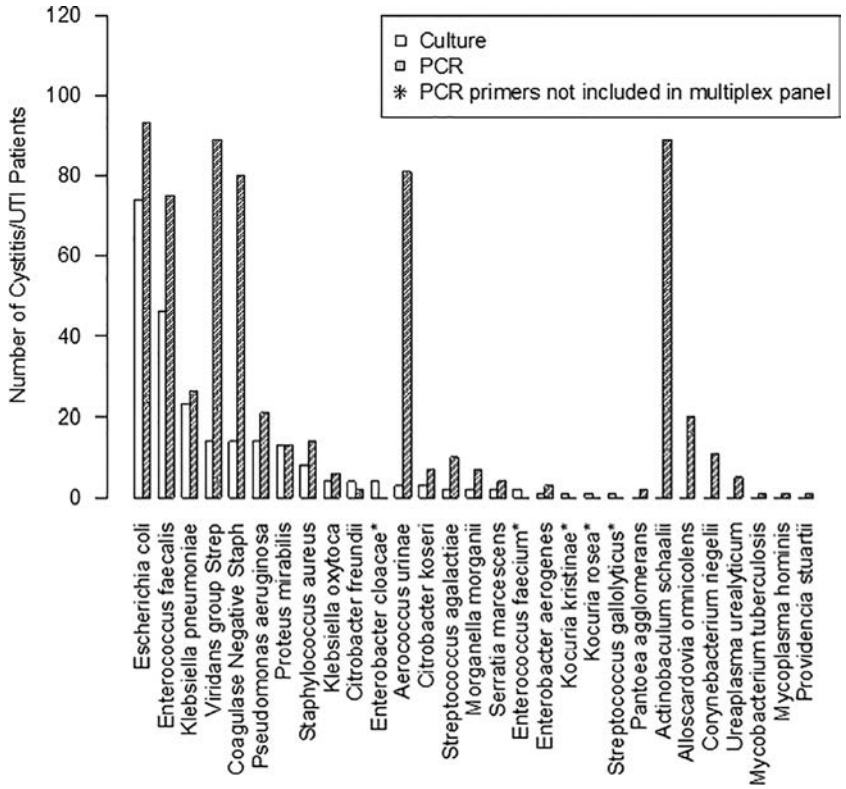

Figure 1. Frequency of detection of bacteria by PCR and urine culture, ordered in decreasing frequency of detection by urine culture.

hours. The rapid, accurate identification offered by PCR can facilitate more appropriate and efficacious treatment and may improve clinical care and outcomes.

UTI is a significant health concern in the United States, causing approximately 7 million visits to a doctor's office, 1 million emergency department visits, and over 100,000 hospitalizations annually, with the cost exceeding $2.6 billion each year.[18,19] Although the patients in this study were outpatients, all of them were at least 60 years old. About 40% of men and 28% of women 70-79 years of age will have nonspecific lower urinary tract symptoms (LUTS) that can be clinical difficult to differentiate from UTI.[20,21] Thus, the diagnosis of UTI on the basis of clinical criteria alone has been reported to have an error rate of approximately 33%.[22] The high numbers of men in this study may reflect that they were enrolled from a busy clinical urology practice that may see more men patients in general. Though women typically have more UTI's than men, the sex differences in this study should not affect the accuracy of PCR vs standard urine culture.

Table 3. Bacteria detected only by PCR and only by culture

Pathogens Detected Only by PCR	Pathogens Detected Only by Culture
Actinobaculum schaalii	*Enterobacter cloacae*
Alloscardovia omnicolens	*Enterococcus faecium*
Corynebacterium riegelii	*Kocuria kristinae*
Mycobacterium tuberculosis	*Kocuria rosea*
Mycoplasma hominis	*Streptococcus gallolyticus*
Pantoea agglomerans	
Providencia stuartii	
Ureaplasma urealyticum	

The bacteria detected only by culture did not have PCR probes in the multiplex PCR panel used for this study.

Polymicrobial infections may be observed in about 39% of UTIs, a potential difficulty in determining appropriate treatment.[14,15] Khasriya showed that urine sediment cultures in patients with LUTS were significantly different than voided urine cultures, and were more commonly polymicrobial, arguing that intracellular bacterial communities and bacteria adherent to the epithelial cells are detected by PCR but not by culture.[23] This may be clinically important, since each pathogen carries a unique pattern of antimicrobial resistance, and one bacterial species can confer antibiotic resistance on other bacterial species in a polymicrobial environment. This emphasizes an important limitation of traditional urine culture: the identification of polymicrobial UTIs is poor, whereas the accuracy of PCR for polymicrobial infections was clearly better. PCR can detect the fastidious organisms that are part of the microbiome; some are clearly pathogens while the role of others is under further investigation.

While other studies have reported higher detection rates using PCR compared to urine culture, most tested against single pathogens, which is clearly not sufficiently comprehensive for clinical use.[24-26] A small number of studies has examined the performance of multiplex PCR, testing for between 9 and 20 pathogens.[10-13] In this study,

our multiplex PCR tested for 31 bacteria and detected 24 different bacteria; the number of bacteria detected exceeded the size of panels used in previous studies.

The importance of multiplexing at this level is underscored by detection rates for polymicrobial infections, defined as 2 or more pathogens. Multiplex PCR was better able to detect polymicrobial infections than culture and was also better able to identify the pathogens. Culture was rarely able to detect cases of >3 pathogens and was unable to identify the bacteria in these cases. In this study culture was unable to identify the pathogens in 85% of polymicrobial infections. Such cases may prove to be clinically significant, inasmuch as mutualism may increase resistance and complicate treatment.

There were a small number of bacteria that were detected by culture but not by PCR. Cases in which culture results were positive and PCR was negative include on 3.6% (21/582) of patients involving 9 bacteria. In 8 of these 21 patients, PCR was negative because the multiplex panel did not include probes for the 5 bacteria infecting those patients. There are limits to multiplexing; it is not currently feasible to test for all known pathogens by PCR. However, judicious selection of PCR targets allows detection and identification of clinically significant bacteria.

On the other hand, PCR detected 661 instances of 24 different bacteria in 56% of patients with about 50% of those being polymicrobial infections. Another advantage of PCR is that since no probe is used to detect lactobacillus, for example, this bacterium, commonly agreed to be a contaminant, is not detected and reported.

E coli was the most common bacteria detected for both PCR and culture. However, the rest of the 5 most frequently detected bacteria were very different between PCR and culture. The rest of the top 5 for PCR were *Actinobaculum schaallii*, *Viridans group Streptococci*, *Aerrococcus urinae*, and *Coagulase negative Staphylococcus*. *Actinobaculum schaalii* was not detected at all by culture. The rest of the top 5 for culture were *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Viridans group Streptococci*, and *Pseudomonas aeruginosa*, all of which were detected by PCR at higher rates than in culture. Others have grown these pathogens from the urine with enhanced urine culture techniques, confirming their importance in the urinary microbiome. Their failure to grow in traditional culture reflects their fastidious nature and further demonstrates the limitations of the traditional culture.

The long-term objective is to determine whether the speed and accuracy of multiplex PCR improves patient care and potentially reduces costs. This study is the necessary first step to confirm that PCR is noninferior to traditional urine culture in identifying bacteria in symptomatic patients. The impact on patient outcomes of this more rapid and accurate organism detection technology will be required to validate the clinical utility before widespread use. Importantly, this multiplex PCR also reports antibiotic resistance for each organism and the susceptibility of the polymicrobial "soup". These data are still being analyzed and will be reported in a separate manuscript. Finally, the clinical impact of the rapid identification of the organism and the resistance are unknown and are the subject an ongoing prospective trial.

A potential weakness of this study is ascertaining the clinical significance of the pathogens identified. The urinary microbiome is still being identified and evaluated; some organisms may be pathogens, some protective and some interdependent. Although many bacteria detected in this study are known pathogens for UTI (eg, *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*),[27] their contribution to pathogenesis may be less definite in cases of polymicrobial infection. That is, UTI symptoms in polymicrobial infections may be caused by a subset of the bacteria, or some combination. Evidence suggests that polymicrobial infections can form cooperative networks that enhance antibiotic resistance, wherein one bacterial species can confer antibiotic resistance on other bacterial species.[28]

A strength of this study is the inclusion criteria for enrolling patients that experienced urologists felt were symptomatic of UTI and required urine culture with or without empiric treatment. This should make the results of this study generalizable to the clinician who is tasked with evaluating and managing the symptomatic patient with clinical UTI symptoms. Another strength is the use of multiplex PCR for detection and identification of polymicrobial infection, allowing for the detection of a larger number and broader range of different bacteria than traditional culture. These data are being further evaluated to examine bacterial interdependence and patterns of bacterial combinations to identify new paradigms of pathogenesis and potential treatment.

CONCLUSIONS

This study shows that multiplex PCR is noninferior to traditional urine culture for detection and identification of bacteria in patients with clinical symptoms of UTI. PCR exhibited greater accuracy than culture for pathogens detected and identified bacteria in 36% of patients who had a negative traditional urine culture. PCR was much more sensitive in detecting polymicrobial infections than urine culture. The accuracy and speed of PCR testing over traditional urine culture, and the potential identification of polymicrobial infections with complicated resistance sharing mechanisms is exciting but requires further study to determine the clinical importance.

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EDITORIAL COMMENT

Standard urine cultures typically result in 48 hours or longer, and in practice this often leads to empiric antibiotic treatment of presumed infections, including the suboptimal use of antibiotics. In one study, 23% of emergency room patients with positive urine cultures received inappropriate empiric antibiotic treatment requiring later intervention following discharge.[1] In the current era of rampant antibiotic resistance, rapid diagnosis of infections and associated antibiotic resistance are desperately needed to expedite targeted treatment, and minimize overuse and inappropriate use of antibiotics. Culture-independent assays, such as the one described in the accompanying paper, may result in as few as 6 hours. The current study is a timely investigation comparing standard urinary cultures and a multiplex PCR-based assay in detecting bacteria in patients with UTI symptoms. The authors conclude that the multiplex PCR assay was noninferior to standard cultures in detection of bacteria in symptomatic patients. This study is a step forward in the implementation of culture-independent assays in the diagnosis and treatment of UTI.

The study raises a number of important points. First, in 22% of cases, multiplex-PCR identified organisms where standard culture was negative. Bacteria uniquely detected by PCR in this study included a number of genera that were also detected in the urinary tract of healthy patients without urinary tract infection in another report.[2] Such findings highlight the importance of further investigation into the urinary microbiome as well as the uropathogenic capacity of nonculturable and other poorly-studied bacteria, and whether their presence in urine should guide therapy. Additionally, the enhanced capacity of PCR-based assays to identify multiple bacterial species will play a particularly important role in the diagnosis and treatment of patients

with complicated UTIs, including subgroups such as chronically catheterized patients or those with prostatitis, in which multiple species in the form of biofilms or intracellular bacterial communities may be present.[3] Indeed, bacteria in such communities exhibit increased rates of resistance.[4]

The authors make note of an arm of the study, as of yet unpublished, designed to describe and analyze the resistance profiles of bacteria identified by multiplex PCR. These results will be critically important in comparing PCR to standard cultures, as the accuracy of the assays in guiding appropriate antibiotic therapy is paramount. Importantly, there is an ongoing prospective, randomized clinical trial wherein both multiplex PCR and standard cultures will be performed on urine samples in patients with UTI symptoms, but the treating physicians will have access to one, the other, or both sets of results (depending on the arm of the study).[5] Outcome measures will include safety, recurrent or persistent infections, and time to symptom resolution. These eagerly awaited results will build on the present study in assessing the clinical efficacy of multiplex PCR relative to traditional cultures in the diagnosis and treatment of UTI, and have the potential to shift the standard of care.

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AUTHOR REPLY

Accumulating evidence shows that Standard Urine Culture (SUC) misses a significant number of potential uropathogens. SUC failure, in part, is due to the incorrect nutrient composition of the growth media, pH, time allotted for growth to occur, and the presence of competing organisms. Additionally, other SUC inadequacies include long turnaround times, false negatives, and the inability to detect more than 2 organisms. The inadequacies may result in treatment failure.

The development of a culture-independent multiplex PCR assay for the identification of uropathogens is an essential step in rapidly and accurately identifying uropathogens. However, the ability of multiplex PCR to identify organisms commonly missed by SUC raises the question of which of these organisms are pathogenic and which is part of the urinary microbiome. Price et al (2018) cite several studies performed in their laboratory demonstrating the "limitations of an Escherichia coli-centric view of UTI" pointing out that the majority of patients in their UTI cohort had additional species present (35 out of 43 patients).[1]

In this paper, we present data showing associations between routinely missed suspect uropathogens and clinically significant urinary tract infections. Reviewers have commented that many of the organisms identified as potential uropathogens can be seen in asymptomatic patients. The converse is also true with many studies demonstrating the presence of common uropathogens, such as *E. coli and E. faecalis* in patients with asymptomatic bacteriuria.[2] The significance of both commonly ascribed uropathogens such as *E. coli* and emerging uropathogens such a *A. baumani* are best viewed as organisms which might be clinically significant in cases of dysbiosis. In these cases, the presence and interaction of these organisms may play an important role in symptom development. This study also underscores high incidence of false-negative cases and inability to identify polymicrobial infections. These failures can lead to treatment failure and increase antibiotic resistance. Hence, the nature of complex UTI infections requires a renewed effort to develop a methodology that identifies the multiple and culture elusive pathogenic organisms and then identify the appropriate antibiotic for all the pathogenic microorganims identified. This complexity has a direct impact on the choice of therapy. In using PCR exclusively, there are difficulties in relying on the presence or absence of resistance genes in determining which therapy is best suited to manage the infections. Since not all antibiotic resistance genes have been identified, there are significant gaps with respect to certain antibiotic modalities. Furthermore, the presence or absence of antibiotic resistance genes could overestimate or underestimate the susceptibility of the resistance pattern.

In that many of these complex infections are polymicrobial in nature, the phenotypic antibiotic response is also impacted by interactions occurring between different bacterial species. The ideal technology must have a quick turnaround time while also detecting slow-growing fastidious, Gram-positive organisms that may impact treatment decisions. This combination of uropathogen identification, coupled with genotypic and phenotypic characterization would allow for a better understanding of this complex process and potentially lead to better therapeutic outcomes.

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