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MULTIPLEX PCR BASED URINARY TRACT INFECTION (UTI) ANALYSIS COMPARED TO TRADITIONAL URINE CULTURE IN IDENTIFYING SIGNIFICANT PATHOGENS IN SYMPTOMATIC PATIENTS

Kirk J. Wojno*[1], David Baunoch*[2], Natalie Luke[2], Michael Opel[2], Howard Korman[1], Colleen Kelly[3], S. Mohammad A. Jafri[1], Patrick Keating[1], Dylan Hazelton[1], Stephany Hindu[1], Bridget Makhloouf[1], David Wenzler[1], Mansour Sabry[1], Frank Burks[1], Miguel Penaranda[2], David E. Smith[2], Andrew Korman[1], Larry Sirls[1]

*Corresponding authors:

Kirk Wojno, M.D., Comprehensive Urology- A Division of Michigan Healthcare Professionals, 31157 Woodward Ave, Royal Oak, MI 48073, kwojno@urologist.org

David Baunoch, Ph.D., Pathnostics, 17661 Cowan, Irvine, CA 92614, dbaunoch@pathnostics.com

Author affiliations:
[1]Comprehensive Urology-A Division of Michigan Healthcare Professionals, 31157 Woodward Ave, Royal Oak, MI 48073
[2]Pathnostics, 17661 Cowan, Irvine, CA 92614
[3]Kelly Statistical Consulting, Palomar Airport Rd, Carlsbad, CA 92011

Abstract

Objective

To evaluate whether multiplex PCR-based molecular testing is non-inferior 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 non-inferiority threshold of 85% (95% CI 85.7-93.6%).

Conclusion

Multiplex PCR is non-inferior 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.

Introduction

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 two 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 four 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 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 saves money. This study is the first step to confirm that PCR is non inferior 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, and are undergoing analysis because the resistance of a single organism is different from the susceptibility results of the polymicrobial "soup", will be reported in a separate manuscript.

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 uL) used to inoculate blood agar plates. A sterile plastic loop (1 uL) 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 (≥ 10^4 CFU/ml), the quantity and morphology of each organism was recorded. The maximum readable colony count using the 1 uL 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 ≥ 10^4 CFU/ml. If ≥ 3 pathogens were present, and one or two were predominant, species

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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 one 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. A 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, 400uL 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 (220uL/well) was added and incubated for 20 minutes at 65C. Proteinase K Mix (PK Mix) was added (50uL/well) and incubated for 30 minutes at 65C. Lysis buffer (125uL/well) and DNA Binding Bead Mix (40uL/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 two-sample t-tests or Fisher's exact tests, as appropriate. Non-inferiority of Guidance™ PCR to traditional culture in terms of detecting bacterial infections was assessed by comparing the lower 95% Wilson-score confidence interval for the positive percentage agreement to the non-inferiority 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 non-inferiority 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 non-inferiority. 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 non-inferiority 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) (Figure 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 bacteria detected (8/217), *Enterobacter cloacae* as monomicrobial infection in 2 patients (1%, 2/217), *Enterococcus faecium* alone in one patient (0.5%, 1/217), *Enterobacter cloacae* and faecium in one patient (0.5%, 1/217), *Enterobacter cloacae* and *E. coli* in one patient (0.5%, 1/217), *Proteus mirabilis* and *Streptococcus gallolyticus* in one patient (0.5%, 1/217), and Kocuria rosea and Kocuria kristinae in one patient each (0.5%, 1/217), (Table 3). Three of these 8 patients tested positive to other bacteria by PCR, one tested positive to Candida albicans and one 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 three 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.

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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. In 3 cases, 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 one 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 two of the culture detected organisms detected by PCR. There were 3 cases in which two pathogens were reported and there was total agreement between PCR and culture. In two cases, the pathogens were *E. coli* and *Enterococcus faecalis;* in one 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 that takes 48 or

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more 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 U.S., causing approximately 7 million visits to a doctor's office, one 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 non-specific 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 versus standard urine culture.

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

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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. This may occur for a number of reasons, but in some cases (5 patients), it occurred because the assay did not include probes for those bacteria. 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. Cases in which culture results were positive and PCR was negative include only 3.6% of patients involving 9 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.

The most common bacterium detected by both PCR and culture was *E. coli* followed by *Actinobaculum schaallii, Viridans group Streptococci, Aerrococcus urinae,* and *Coagulase negative Staphylococcus. Actinobaculum schaalii* was not detected by culture at all and the other four bacteria were rarely detected by 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

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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 saves money. This study is the necessary first step to confirm that PCR is non inferior 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 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 (e.g., *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. This data is 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 non-inferior 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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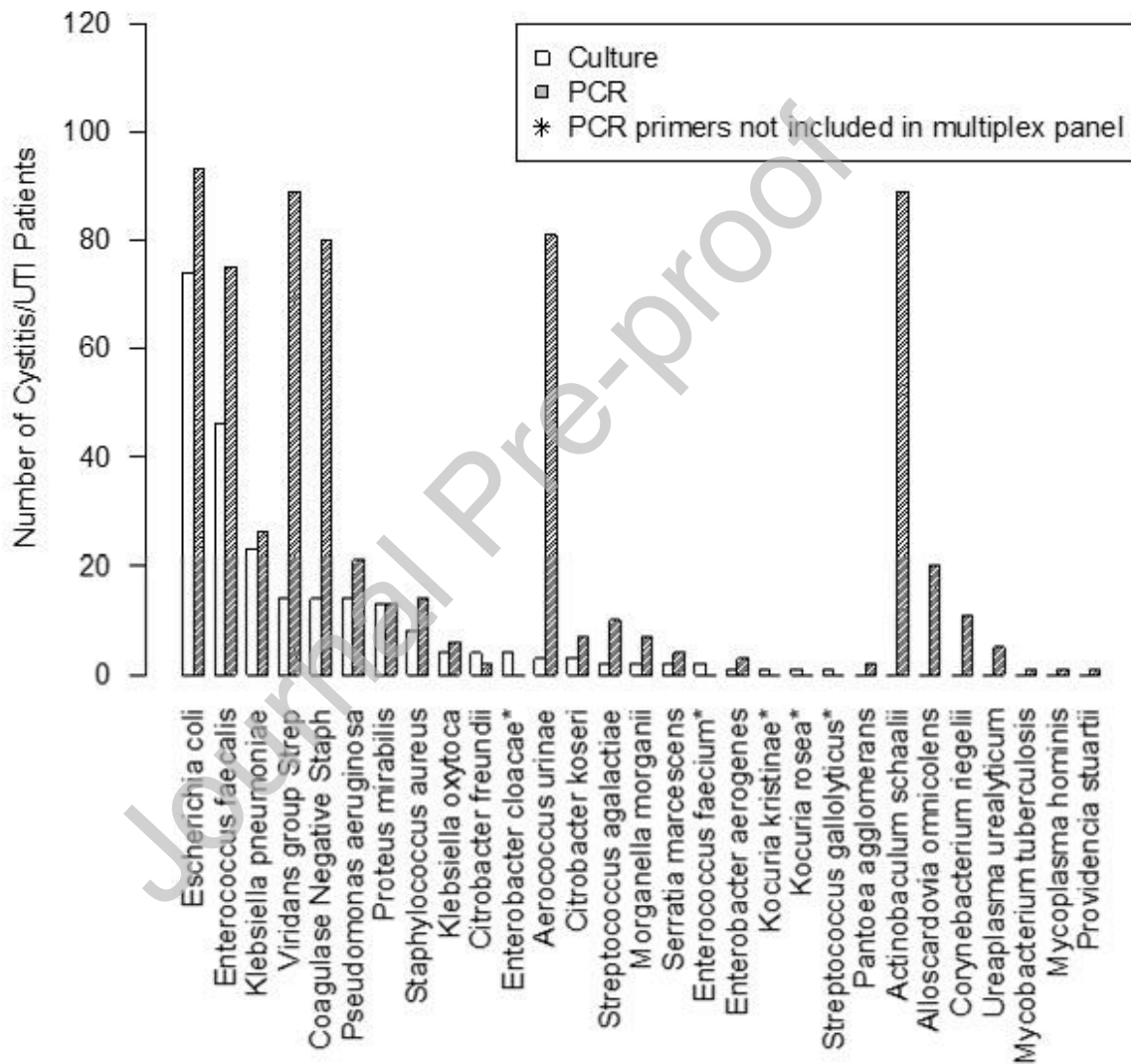
Figure 1. Frequency of detection of bacteria by PCR and urine culture, ordered in decreasing frequency of detection by urine culture.

Table 1. Study Participant Demographics and Symptoms

Parameter	Total	Male	Female	p-value [1]
Age (years)				
n	582	347	235	
Mean (SD)	77.2 (7.9)	76.9 (7.9)	77.7 (7.9)	0.01
Median	77	76	79	
Min, Max	60, 95	60, 95	60, 95	
Symptoms – n (%)				
Dysuria	219 (37.6%)	110 (31.7%)	109 (46.4%)	0.0005
Urinary incontinence	196 (33.7%)	145 (41.8%)	51 (21.7%)	< 0.0001
Cloudy or Strong-smelling urine	132 (22.7%)	75 (21.6%)	57 (24.3%)	0.48
Pain	18 (3.09%)	7 (2.02%)	11 (4.68%)	0.09
Abdominal	7 (1.20%)	3 (0.86%)	4 (1.70%)	0.45
Flank	5 (0.86%)	1 (0.29%)	4 (1.70%)	0.16
Lower back	4 (0.69%)	1 (0.29%)	3 (1.28%)	0.31
Penis / scrotum	2 (0.34%)	2 (0.58%)	0 (0.00%)	1.00
Pelvic discomfort	24 (4.12%)	6 (1.73%)	18 (7.66%)	0.001
Lower grade fever	1 (0.17%)	1 (0.29%)	0 (0.00%)	1.00
Agitation	2 (0.34%)	2 (0.58%)	0 (0.00%)	0.52
Frequency	10 (1.72%)	4 (1.15%)	6 (2.55%)	0.21
Nocturia	1 (0.17%)	0 (0.00%)	1 (0.43%)	0.40
Patient-reported Hematuria	1 (0.17%)	0 (0.00%)	1 (0.43%)	0.40
Dipstick Results – n (%)				
Hematuria	246 (42.7%)	138 (40.4%)	108 (46.2%)	0.17
Atypical Symptoms – n (%)				

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Parameter	Total	Male	Female	p-value [1]
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%)	< 0.0001

[1] 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. The agreement between PCR and urine culture for positive cultures was 196/217 (90%), exceeding the non-inferiority threshold. Polymicrobial infections are those having ≥ 2 organisms infections. PCR was significantly more sensitive than urine culture for detecting polymicrobial infections.

			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		67 (11.5%)	63 (10.8%)	130 (22.3%)	235 (40.4%)	365 (62.7%)	235/365 (64.4%)
	Total		166 (28.5%)	160 (27.5%)	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%)	

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%)

Table 3. Bacteria detected only by PCR and only by culture. The bacteria detected only by culture did not have PCR probes in the multiplerx PCR panel used for this study.

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	