Accuracy of molecular diagnostic techniques in patients with a confirmed urine culture: A systematic review and meta-analysis

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Abstract

Introduction: We aimed to identify the molecular diagnostic techniques available for urinary tract infection (UTI) diagnosis and their accuracy compared to traditional urinary culture.
Methods: A systematic search was performed in MEDLINE (OVID), EMBASE, LILACS, and the Cochrane Central Register of Controlled Trials (CENTRAL). The populations were adult and pediatric patients with confirmed UTI by reference standard urine culture. The index test for the diagnosis of UTI was any molecular diagnostic technique. The primary outcome was the diagnosis of UTI with measures of sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), positive likelihood ratio (LR+), negative likelihood ratio (LR–), diagnostic odds ratio (DOR), and area under the curve. The operative characteristics were determined, and a meta-analysis was performed. The evaluation of each included study was performed with the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool.
Results: We identified 1230 studies with the search strategies. Ultimately, 13 studies met the inclusion criteria for qualitative analysis, and seven were included for the meta-analysis. Four molecular techniques were identified; however, it was only possible to synthesize the

information from two of them. In multiplex polymerase chain reaction (PCR) meta-analysis, overall sensitivity was 0.80 (95% confidence interval [CI] 0.73–0.86) and specificity was 0.83 (95% CI 0.52–0.95). For the DOR, the overall result was 21 (95% CI 4.8–95). For reverse transcription (RT)-PCR, sensitivity was 0.94 (95% CI 0.73–0.99) and specificity was 0.59 (95% CI 0.063–0.96). For the DOR, the overall result was 23 (95% CI 1.1–467).

Conclusions: Multiplex PCR and RT-PCR are molecular techniques that might be comparable to standard urine culture for UTI diagnosis. Refinement of these new diagnostic tools will avoid unnecessary antimicrobial therapy and the consequent development of drug-resistant resistant pathogens, as well as improve the ability to identify patients at risk and prevent or minimize sequelae derived from the infection.

Introduction

Lower urinary tract symptoms (LUTS) are exceedingly prevalent among adults (1), and 60-80% of these episodes are related to significant bacteriuria (2), being an indicator of either bacterial colonization or infection of the urinary tract (3).

Urinary tract infection (UTI) is among the most prevalent infections, affecting near 50% of the population at least once in their lifetime (4). The initial diagnostic examination begins with clinical symptoms (4) and microbiology techniques to support medical decisions. Urine is cultured on agar plates, and antimicrobial susceptibility testing (AST) is performed (4).The gold standard is the direct detection of the pathogen itself in clinical samples (5).

A $\geq 10^5$ CFU/mL threshold has high specificity for a UTI, but sensitivity is only nearly 50% (1). Nonetheless, it was recently confirmed that low counts of E. coli in midstream urine were highly predictive for its presence in the bladder and not caused by contamination (6). Indeed, lowering the threshold to $\geq 10^3$ increases sensitivity, with minimal reductions in specificity (1). Despite that, 25-30% of these symptomatic women will have a negative urine culture (2).

Recent evidence suggests that UTIs are not limited to a superficial luminal infection (1). Conversely, intracellular bacterial communities (ICB) have been described (7), complicating the interpretation of the culture-based diagnosis as bacteria are then undetected by standard urine cultures (1). Based on these acknowledgments, innovative methods of identification of uropathogens have emerged (1), increasingly relying on molecular techniques (5).

To date, there are no systematic reviews on the use of molecular diagnostic techniques in patients with suspected urinary tract infections. We aimed to determine the

diagnostic accuracy of novel molecular tools compared to traditional urinary culture to diagnose UTI in patients with lower urinary tract symptoms.

Methods

This systematic review was performed according to the recommendations of the Cochrane Collaboration and following the PRISMA-P Statement.

Eligibility criteria

We included clinical trials, cohort, case-control, and cross-sectional studies providing data on accuracy for diagnosis of urinary tract infection in adult and pediatric patients.

The index test for the diagnosis of urinary tract infection was any molecular diagnostic technique. The reference standard was urine culture.

Studies were included if they fulfilled the following criteria: (1) reference standard for urinary tract infection represented by urine culture performed before/ after any molecular diagnostic technique; (2) availability of many nondiagnostic urine cultures; and (3) availability of many diagnostic urine cultures classified as true positives (TPs), false positives (FPs), false negatives (FNs), and true negatives (TNs) either as group totals or by case-by-case enumeration of diagnoses.

The primary outcome was the diagnosis of urinary tract infection with measures of sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR–), diagnostic odds ratio (DOR), and area under the curve.

For all outcomes, no time specification was necessary.

We excluded studies that did not meet the criteria described above, non-related to urinary tract infection, non-related to a diagnostic method, flow-cytometry studies, animal studies, non-bacterial urinary infections, genitourinary tuberculosis infection, and neurogenic patients. Also, were excluded articles that could not be found or whose full-text were not available.

Information sources

A systematic search of studies was performed in the following databases from inception to nowadays: MEDLINE (OVID), EMBASE, LILACS, and the Cochrane Central Register of Controlled Trials (CENTRAL) (Appendix 1). We scanned references from relevant articles identified through the search, conferences, thesis databases, Open Grey, Google scholar, and clinicaltrials.gov to ensure literature saturation. There were no setting or language restrictions.

Study selection and data collection

Two reviewers (X.G. and K.O.) independently evaluated the systematically searched titles and abstract. They scanned full texts of relevant studies, applied pre-specified inclusion and exclusion criteria, and extracted the data. Disagreements were resolved by consensus

between two reviewers, and where disagreement could not be solved, a third reviewer (H.G.) was consulted to dissolve conflict.

Two trained reviewers using a standardized form independently extracted the following information from each article: study design, year of publication, geographic location, authors names, title, number of patients included, timing, variables, interventions, outcomes, and association measures.

Risk of bias

We used the QUADAS2 tool to assess the risk of bias in these studies.

Data analysis/synthesis of results

The statistical analysis was performed using R and Review Manager 5.3 (RevMan® 5.3). For the outcomes, information about sensitivity, specificity, likelihood ratios, and diagnostic odds ratios was reported based on concordance between molecular diagnostic techniques results and urinary cultures. The results are displayed in forest plots of the estimated effects of the included studies with a 95% confidence interval (95% CI), and we pooled the information with a random effect meta-analysis according to the heterogeneity expected.

Publication bias

An evaluation was conducted to identify reporting or publication bias using the funnel plot.

Sensitivity analysis

We performed sensitivity analysis extracting weighted studies and running the estimated effect to find differences.

Analysis by subgroup

A subgroup analysis was performed for the qPCR and Multiplex-PCR.

Results

Selection of studies

In the initial search, a total of 1230 studies were found. After the initial filter by full text, 13 studies met the inclusion criteria: Lehmann LE. 2011 (8); McDonald M. 2017 (9); DeMarco ML. 2014 (10); Ishihara T. 2020 (11); Wojno KJ. 2020 (12); Guzman-Puche J. 2019 (13); Raja B. 2017 (14); Felt JR. 2017 (15); Montgomery S. 2017 (16); Tchesnokova V. 2017 (17); Chapelet G. 2016 (18); Burillo A. 2014 (19); Lehmann LE. 2010 (20) and seven were included for the meta-analysis (Figure 1).

Characteristics of included studies

The studies were published between 2010 and 2020. Seven studies were performed in the US, six in Europe, and one in Japan.

The total number of participants was 3995, with an average number of participants per a study of 285 (10-958). The age range was between <28 days and 104 years, and most individuals were women (64%).

The definition for a positive urine culture varied between $<10^3$ CFU/ML to $\geq 10^5$ CFU/ML; the most used molecular technique was qPCR (43%), and the top frequent germ isolated irrespective of the method was E. coli (Table 1).

Risk of bias assessment

All the included studies were evaluated with a low risk of bias concerning the reference standard, mainly regarding flow and timing. However, most of the studies had an unclear risk about the index test because they did not specify the threshold used for the diagnosis or its standard test execution. As for the selection of patients, two studies had a high risk of bias, one because it included both symptomatic and asymptomatic patients and the other one because of the exclusion of patients with fever and acute pyelonephritis (Figure 2a and 2b).

Results of the individual studies

Diagnosis of UTI with RT-PCR

Regarding this molecular technique, we pooled five studies: Felt JR. 2017 (15); Tchesnokova V. 2017 (17); McDonald M. 2017 (9); Chapelet G. 2016 (18); Wojno KJ. 2020 (12).

The bivariate random-effects model and estimation of SROC curves indicated that the overall sensitivity was 0.94 (95% CI 0.73 to 0.99) and that the overall specificity was 0.59 (95% CI 0.063 to 0.96). For the DOR, the overall result was 23 (95% CI 1.1 to 467) (Figures 3a; Table 2).

Diagnosis of UTI with multiplex PCR

Regarding multiplex PCR, we included two studies: Lehmann LE. 2010 (20) and Lehmann LE. 2011 (8).

The bivariate random-effects model and estimation of SROC curves indicated that the overall sensitivity was 0.80 (95% CI 0.73 to 0.86) and that the overall specificity was 0.83 (95% CI 0.52 to 0.95). For the DOR, the overall result was 21 (95% CI 4.8 to 95) (Figures 3b; Table 2).

Multiple techniques
The remaining studies had other diagnostic methods, including MALDI-TOF MS Mass Spectrometry, Next-Generation DNA Sequencing (NGS) technology-based upon Bacterial 16S rRNA amplicon sequencing analysis- a Panel of Recombinase Polymerase Amplification Assays and Narrow-Angle Forward Laser Light Scattering technology (10,11,13,14,16,19).

Three studies evaluated MALDI-TOF MS Mass Spectrometry with a sample size of 1378 and had variable sensitivity ranging from 67% to 92% and specificity above 70% to 100%. Only the two studies performed in Spain reported the characteristics of the population, all adults and primarily female; the facility was variable.
Only one study assessed Next-Generation DNA Sequencing (NGS) technology, no cut-off value for UTI diagnosis by standard urine culture was reported, and only ten patients participated. Most of them had upper and complicated UTI (80% and 70%, respectively), the overall sensitivity was 100%; however, specificity was not able to calculate since there were no true negatives.

Also, only one study reviewed Narrow-Angle Forward Laser Light Scattering technology in a pediatric population. They included 439 patients and presented a sensitivity of 96% with a specificity of 71%; no socio-demographic characteristics were recorded (Table 1).

Discussion

Summary of the primary outcomes
Both multiplex PCR and RT-PCR showed high overall sensitivity, specificity, and diagnostic odds ratio.

Contrast with literature
At present, urine culture continues to be the gold standard for the diagnosis of UTI. However, although specificity is relatively high, sensitivity remains a pitfall since almost one-third of symptomatic patients have negative test results (1,2,4).

Even if urine culture remains the benchmark, there are several disadvantages as it carries essential contamination rates and high thresholds, which may miss relevant infections (9).

Since no etiology for LUTS is found in patients with standard negative urine cultures, treatment of such patients is unfortunately targeted at symptom management based on classifications as syndromic entities and diagnoses of exclusion such as overactive bladder (OAB) and interstitial cystitis/bladder pain syndrome (IC/BPS) according to their primary complaint. It might affect quality of life because of its low possibilities of complete recovery and resolution of symptoms, with pauper hope of a definitive cure (1).

Additionally, the time needed to obtain a definitive result in traditional culture tests is usually ≥24 hours, so prolonged turnaround times obligates the physician to initiate aggressive empiric antibiotic before (or with no) pathogen identification. Each hour of delay in antimicrobial administration in patients with septic shock is associated with a mean 8% decrease in survival rate (20).

With this in mind, the most significant benefit of molecular diagnostic techniques is the faster pathogen identification which allows an earlier selective antimicrobial therapy (8). However, PCR methods somehow limit microorganism detection because pathogens that are not included in the settings panel will be restricted. Besides, these techniques detect important or dead pathogens and DNA fragments from degraded pathogens, unlike urine cultures that exclusively detect viable and reproductive organisms (8). Interpretation of positive PCR findings in the absence of clinical UTI signs is unclear, but it might suggest a passed or subclinical infection (8).

In the context of increasing clinical symptoms, ongoing indisposition, and risk of ascending infections or urosepsis, choosing an initially inappropriate antimicrobial contributes to certain levels of morbidity and mortality if UTU is undiagnosed or untreated (3,4). Nonetheless, the problem of increased resistance reduces the chance of efficient prophylaxis and treatment (9).

A significant limiting factor of molecular techniques to bear in mind is that microbiological cultures are still, to the date, the only way to the acquisition of an antibiogram with sensitivity test necessary for ongoing treatment (8,20,21). Therefore antibiotic therapy triggered by PCR results might be insufficient due to undetected antibiotic resistance (8,20).

The investigation has led to the search for innovative methods of identifying uropathogens aiming to improve diagnostic performance that hopefully will impact the understanding of the physiopathology and control of IBCs (3–5).

Screening tests should be easy to use, cost-effective, and amenable to point-of-care testing. They must deliver pathogen-positive or pathogen-negative results in minutes and AST within a few hours of sample collection. This efficiency would obviate the initiation of empirical antibiotics without pathogens and facilitate pathogen-specific antibiotic selection (4).

Even if PCR methods cannot yet replace the traditional microbiological urine culture by itself additionally, PCR detection of resistance genes as surrogate parameters for antibiotic resistance is practicable (8). It can then supplement urine culture and reduce the time needed to decrease clinical symptoms and resistant pathogens (8,20,21).
The greatest obstacle to implementing PCR technology into clinical practice is perhaps financial feasibility. Notwithstanding the costs of that PCR-based test are significantly higher than current methods, it must be taken into account the savings that might result

from a faster and more accurate diagnosis, including decreasing the length of hospitalization and preserving hospital resources (15,21).

To date, there are no systematic reviews nor meta-analyses on the use of molecular diagnostic techniques in patients with suspected urinary tract infection to contrast with the present study.

Strengths and limitations

The strengths of this analysis included the selection of patients, the reference standard, and the flow and timing, as most were with a low risk of bias related to these aspects. Additionally, to our knowledge, this is the first study assessing this topic. One of the significant limitations of the analysis was the unclear risk of bias regarding the index tests. The false-positive rate is another critical limitation of molecular diagnostic techniques. These are relevant when finding urinary symptoms and negative cultures. Although tempting and promising, they cannot wholly replace standard diagnostic methods. Nowadays, they serve as adjuvants to traditional urine cultures, considering the scarce published literature and the limitations of the technique to overcome. Consequently, these results must be interpreted cautiously.

The test can be repeated to overcome the false positive rate issue. In some cases, a test is performed three times, and the patient is declared positive only if two out of the three tests are positive. Accordingly, we might not consider these molecular tests confirmatory but a screening test.

Conclusions

Multiplex PCR and RT-PCR are molecular techniques comparable to standard urine culture for the diagnosis of UTI. Refinement of these new diagnostic tools will avoid unnecessary antimicrobial therapy and consequent development of drug-resistant resistant pathogens and improve our ability to identify patients at risk and prevent or minimize sequelae derived from the infection.

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Figures and Tables

Figure 1. Flowchart of selected studies.

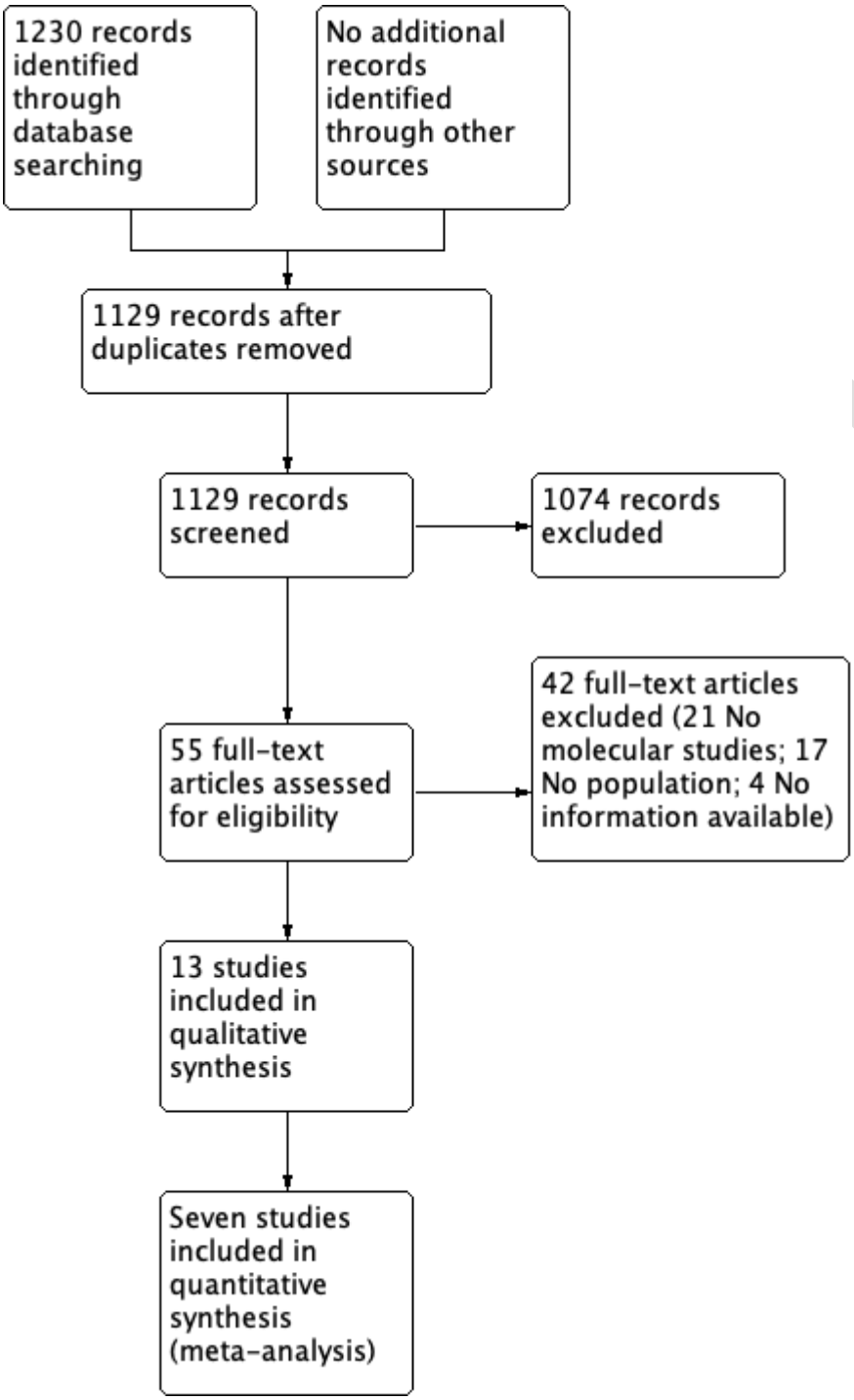

Figure 2. Risk of bias assessment **(A)** within studies, and **(B)** across studies.

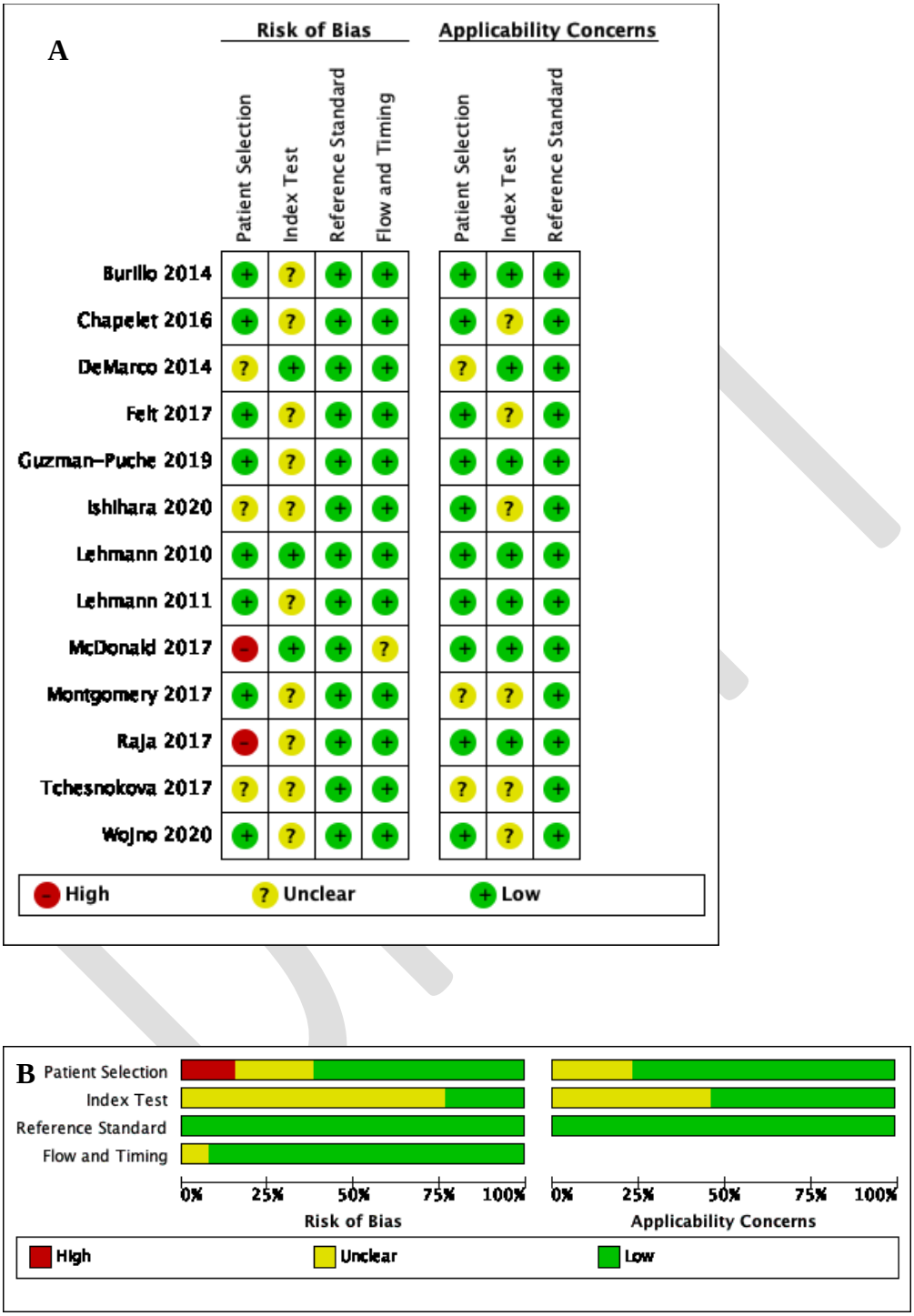

Figure 3A. ROC curve for diagnosis of urinary tract infection with reverse transcription polymerase chain reaction (RT-PCR).

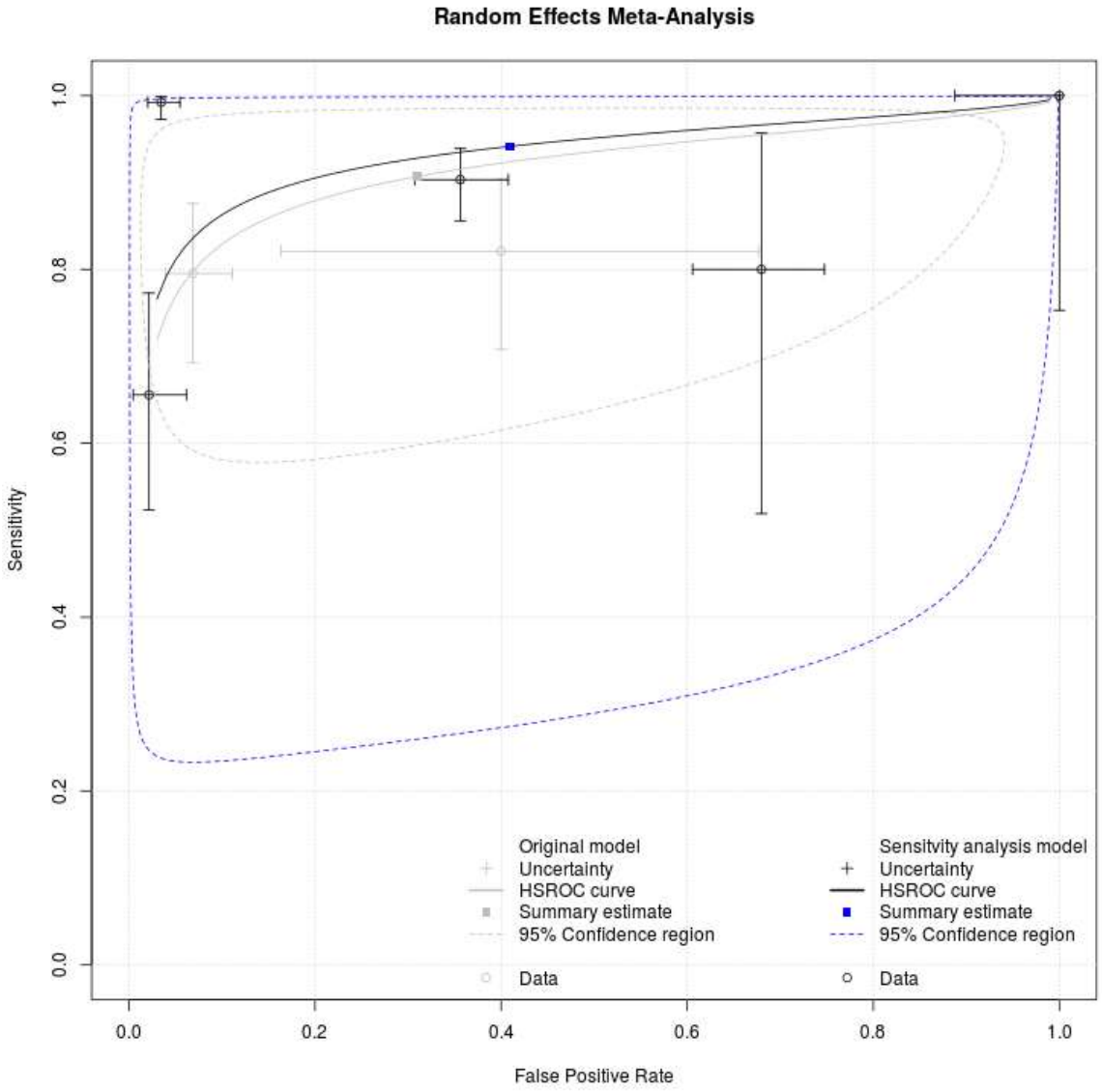

Figure 3B. ROC curve for Diagnosis of urinary tract infection with multiplex polymerase chain reaction (PCR).

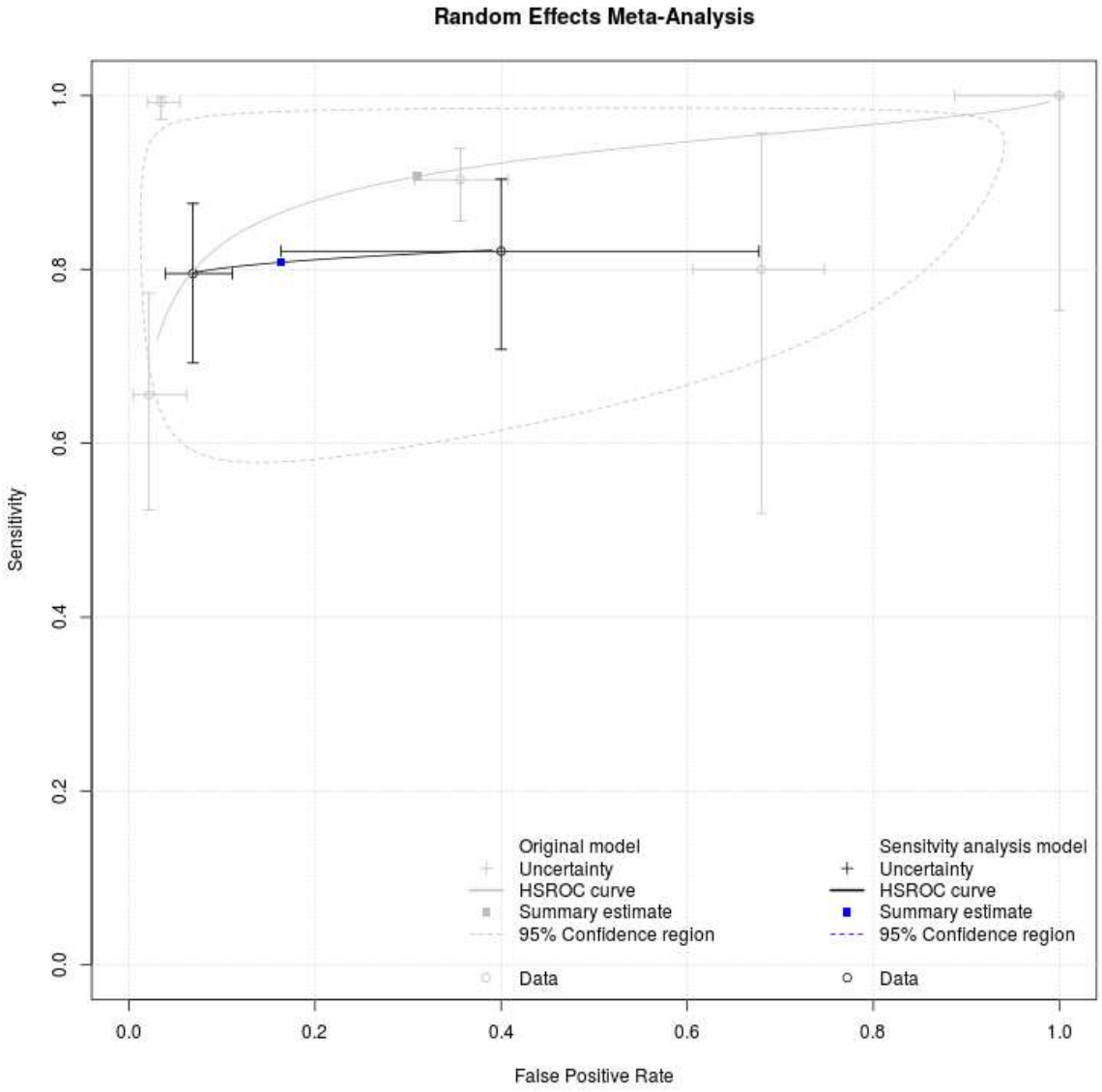

Table 1. Characteristics of included studies

Autor, year	Study type	Time	Setting	No. subjects	No. samples	Age	Female	L-UTI	U-UTI	C-UTI	UN-UTI	Out-patient	ICU	Urine collection method	Positive culture (cfu)	Culture agar plate	Molecular test	Tradename of molecular test	Most frecuent germ by culture and molecular test
Lehmann LE, 2011	Cross-sectional	NR	Germany	81	82	Mean 49 /range (18–79)	43 (53%)	39 (48%)	43 (52%)	NR	NR	NR	NR	Midstream URINE	$>10^5$ CFU/ml	Cled-, macconkey-agar, and malt extract agar	RT-PCR	SEPTIFAST®	E. coli
McDonald M, 2017	Clinical trial	Jan to Dec 2016	United Sates	44		NR	29 (66%)	44 (100%)	0 (0%)	19 (43%)	25 (57%)	NR	NR	Midstream urine	$>10^5$ CFU/ml	NR	RT-PCR + DNA pyrosequencing method	Microgen dx-decodextm	NR
DeMarco ML, 2014	Cross-sectional	NR	United Sates	100	100	NR	NR	NR	NR	NR	NR	NR	NR	NR	$\geq 10^5$ CFU/ml	Sheep blood agar and macconkey agar	Diafiltration MALDI-TOF MS	NR	NR
Ishihara T, 2020	Case-series	Jan 2017 to Dec 2017	Japan	10	10	Median 85/range (66–89)	7 (70%)	2 (20%)	8 (80%)	7 (70%)	3 (30%)	6 (60%)	3 (30%)	NR	NR	NR	NGS methodology (bacterial 16s RRNA amplicon sequencing analysis)	Ionpgm	E. Coli
Wojno KJ, 2020	Cross-sectional	March to July 2018	United States	582	582	Mean 77/range (60–95)	235 (40%)	NR	NR	NR	NR	582 (100%)	NR	Clean catch or catheterization	$\geq 10^4$ CFU/ml	Blood agar plates, colistin and nalidixic acid agar/macconkey agar plates	Multiplex-PCR	Guidance UTI test	E. Coli
Guzman-Puche J, 2019	Cross-sectional	NR	Spain	320	320	Men mean 60.6±20.4	277 (86.6%)	NR	NR	NR	NR	258 (80.6%)	NR	Nr	$>10^4$ CFU/ml	Cysteine lactose electrolyte deficient (cled) agar	MALDI- TOF MS	MALDI- TOF microflex lt mass spectrometer	E. Coli
Raja B, 2017	Retrospective cohort	Aug 5 to 18, 2014	United States	50	50	Median 80.5/range (13–104)	35 (70%)	NR	NR	NR	NR	NR	NR	NR	$>10^4$ CFU/ml	SHEEP BLOOD AGAR PLATES	Panel of recombinase polymerase amplification assays	RPA	E. Coli
Felt JR, 2017	Prospective cohort	Sept 2015 to June 2016	United States	200	193	9 months (IQR 5–13.5)	135 (70.5%)	NR	NR	NR	NR	132 (68.4%)	NR	Bladder catheterization or suprapubic needle aspiration	$>10^4$ CFU/ml	Nr	RT-QPCR	NR	E. Coli
Montgomery S, 2017	Prospective cohort	August to Sept 2016	United States	439	439	7 years (IQR 3–15)	NR	NR	NR		NR	NR	NR	Clean-catch or catheterization	$>10^4$ CFU /ml	Sheep blood and macconkey agar plates	Narrow-angle forward laser light scattering	Bacterioscan 216dx	E. Coli
Tchesnokova V, 2017	Prospective cohort	July 2014 to Nov 2015	United States	750	750	Mean 52.5/range (18–90)	615 (82%)	NR	NR	NR	NR	NR	NR	NR	$>10^4$ CFU /ml	Nr	QPCR-based test 7-SNP test	NR	E. Coli
Chapelet G, 2016	Prospective cohort	March to Nov 2014	France	200	200	Mean 44.5±23.0	155 (77.5%)	31 (15.5%)	124 (62%)	NR	NR	NR	NR	NR	$\geq 10^3$ CFU /ml	Chromogenic agar culture	Triplex real-time PCR	Asec rapid test	E. Coli
Burillo A, 2014	Prospective cohort	May to June 2012	Spain	958	1000	Median 60.2 (IQR 41.2–76.3)	544 (58.6%)	NR	NR	NR	NR	25.8%	NR	Midstream voided, bladder catheterization	$\geq 10^5$ CFU ml	Cystine lactose-electrolyte-deficient (cled) agar (bio-me rieux, marcy l'etoile, france)	MALDI-TOF MS mass spectrometry	NR	E. Coli
Lehmann L.E, 2010	Prospective cohort	NR	Switzerland	189	301	Median 66 /range (18–97)	96(50.7%)	76 (40.2%),	83 (43.9%)	NR	NR	NR	100	NR	NR	Cled, macconkey and malt extract agar	RT-PCR	NR	E. Coli

Table 2. Diagnosis of UTI with RT-PCR						
	RT-PCR			**Multiplex PCR**		
Parameter	**Estimate**	**Lower**	**Upper**	**Estimate**	**Lower**	**Upper**
Sensitivity	0.941	0.731	0.990	0.808	0.734	0.866
Specificity	0.591	0.063	0.969	0.837	0.527	0.959
False positive rate	0.409	0.031	0.937	0.163	0.041	0.473
Diagnostic odds ratio	23 222	1152	467 969	21 625	4888	95 680
Likelihood ratio positive	2300	0.387	13 661	4957	1403	17 515
Likelihood ratio negative	0.099	0.017	0.562	0.229	0.158	0.333

RT-PCR: reverse transcription polymerase chain reaction; UTI: urinary tract infection.