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**Review – Infections****Molecular Diagnostic Methods Versus Conventional Urine Culture for Diagnosis and Treatment of Urinary Tract Infection: A Systematic Review and Meta-analysis**Alec Szlachta-McGinn^a, K. Marie Douglass^a, Un Young Rebecca Chung^b, Nicholas James Jackson^b, J. Curtis Nickel^c, A. Lenore Ackerman^{a,d,*}^aDepartment of Obstetrics and Gynecology, Division of Female Pelvic Medicine and Reconstructive Surgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA; ^bDepartment of Medicine Statistics Core, University of California Los Angeles, Los Angeles, CA, USA; ^cDepartment of Urology, Queens University, Kingston, Canada; ^dDepartment of Urology, Division of Pelvic Medicine and Reconstructive Surgery, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA**Article info****Article history:**

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Keywords:Urinary tract infection
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specificity in controls. Additional data comparing patient symptoms and cure rates following antibiotic selection directed by molecular methods compared with culture are needed to elucidate their place in UTI care.

Patient summary: We compare culture-independent molecular methods with urine culture in the management of urinary tract infection. We found good evidence that molecular methods detect more bacteria than culture; however, the clinical implications to support their routine use are unclear.

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1. Introduction

Urinary tract infection (UTI) is one of the most common types of bacterial infections in adults [1,2]. An estimated 60% of women will experience at least one UTI in their lifetime [3]. UTI treatment and management cost billions of health care dollars annually in both the ambulatory and the inpatient setting [3,4]. Molecular testing for the diagnosis of UTI, including next-generation sequencing (NGS) and polymerase chain reaction (PCR), has increased in popularity recently owing to frustration with using conventional urine culture. Molecular diagnostic methods advertise increased sensitivity in the detection of urinary pathogens, which make their use attractive to patients and providers; however, efficacy in this setting is as yet unclear.

Conventional culture has sensitivity of only approximately 60% in detecting acute UTI [5]. The traditional threshold used to diagnose infection is 10^5 colony-forming units (cfu) per milliliter [6,7]. This threshold derived from a study in the 1950s attempting to distinguish contamination from true bacteriuria: 10^5 cfu/ml on a voided sample was best able to predict bacteriuria on catheterization [8,9]. More recent evidence, however, suggests that lower colony counts, as low as 10^2 cfu/ml, may be indicative of cystitis in acutely symptomatic women [10–13], calling into question how to manage positive cultures with colony counts between 10^2 and 10^4 cfu/ml. Additionally, conventional urine culture selects for bacteria that are mainly aerobic and fast growing, such as *Escherichia coli*, *Enterococcus*, and *Staphylococcus* species [14,15]. The majority of human commensal bacteria are slow growing, anaerobic, or fastidious, or do not grow well in conventional cultures, so their role in UTI pathophysiology is unclear [16].

With the development of molecular methods of microbial community profiling, such as NGS and PCR, it is now known that the bladder harbors a diverse range of bacterial inhabitants, even in healthy, asymptomatic individuals [5,15,17–21]. NGS is a culture-independent technique to identify the microorganisms of a given sample, circumventing some of the limitations of conventional urine culture [15]. NGS typically refers to amplicon sequencing in which targeted primers are used to amplify a region of DNA (typically a region of the 16S rRNA locus); the resulting sequences are compared with bacterial sequence databases to allow identification of the taxa present. Shotgun sequencing or metagenomics is another deep sequencing approach in which all the DNA in a sample is fragmented and sequenced, then reassembled into genomes that represent the different

organisms present in a sample; for urine, this might include DNA from bacteria, viruses, fungi, as well as the human host [15,19]. Multiplex PCR refers to the use of pathogen-specific primer probes to determine the taxa present [22]. Primers target a conserved region of the microbial genome to allow microbial identification at the genus or species level after template amplification [22]. In contrast to NGS, PCR is able to detect only taxa targeted by preselected primers [22]. Qualitative PCR determines whether a pathogen is present or absent, whereas quantitative PCR determines the amount of pathogen present. These methods have transformed our understanding of the urinary microbiome and implicated these more complex bacterial communities in the etiology of UTI symptoms [15,19,23].

In the past decade, these molecular diagnostic approaches have been used in research to identify microbes in the urine of patients with UTI that is not cultivable with conventional urine culture [5,14,17,18,20,21,24] leading to current availability of several commercial culture-independent diagnostic services for use in clinical practice (NGS and PCR) [25,26]. This systematic review and meta-analysis aims to compare the use of culture-independent molecular-based diagnostic technologies with conventional urine culture in the diagnosis and treatment of symptoms of UTI.

2. Evidence acquisition

This systematic review was registered at Prospero (CRD42021270636, <https://www.crd.york.ac.uk/PROSPERO/>) and was exempted from institutional review board approval. The authors employed the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) checklist in creating and conducting this review and meta-analysis. The online systematic review management tool Covidence was used for abstract screening, full-text review, and data extraction.

The study outcomes, experimental and comparison groups, inclusion and exclusion criteria, and analytical approach were defined prior to the literature search and registration on Prospero. The primary outcome in this review was defined as the comparison of molecular diagnostic technologies with conventional urine culture in the diagnosis and treatment of UTI. Molecular methods included NGS (including 16S rRNA amplicon sequencing and shotgun sequencing) and quantitative or qualitative PCR. The secondary outcome was defined as the use of molecular diagnostic technologies to guide antibiotic ther-

apy through assessment of the genotypic and phenotypic resistomes (profile of antibiotic resistance). Data collected from each publication as well as inclusion and exclusion criteria for this systematic review and meta-analysis are listed in [Supplementary Table 1](#). One researcher (A.S.) performed a literature search on Embase, Ovid, and PubMed ([Supplementary material](#)). The references of studies that met the inclusion criteria and other review articles were screened to identify additional articles for inclusion in the review. The search engine Embase was used to search the available gray literature. The initial literature search was performed on July 31, 2021. A second search using the same queries was conducted on August 23, 2021, and a third literature search was performed on February 12, 2022. Two authors (A.S. and K.D.) independently reviewed each study identified through the literature search. Data were extracted by a single reviewer (A.S.). Conflicts at all stages were settled by a third reviewer (A.A.). Automation tools were not utilized in the screening process.

The effect measures for the primary outcome were defined: species diversity using the Shannon Diversity Index (H) and species similarity using Sørensen's coefficient of similarity. To evaluate for differences between the aggregate number of positive and negative tests using each diagnostic method, the chi-square test was used. The Shannon Diversity Index was compared by calculating the difference in H between culture and molecular method for each study. Aggregate effects were computed based on a meta-analysis using a random-effect restricted maximum likelihood model. The effect measures for the secondary outcome were defined: genotypic resistome determined by molecular methods, phenotypic resistome determined by conventional urine culture, type of antibiotic(s) prescribed using either method, and comparison of patient symptom response to antibiotics through the use of standardized questionnaires. All results compatible with each outcome were included in this review.

Risk of bias and methodological quality were assessed independently by two authors (A.S. and K.D.) using the Cochrane risk of bias tool and the Newcastle Ottawa Scale [27,28]. Based on these assessments, each study was graded as having a high, a low, or an indeterminate risk of bias. The Grades for Recommendation, Assessment, Development, and Evaluation (GRADE) system was used to assess the quality of evidence for each outcome by considering the risk of bias, imprecision, consistency, indirectness, and publication bias. Four possible quality of evidence ratings were assigned: high, moderate, low, and very low. Statistical heterogeneity of each study was calculated using the I^2 statistic.

3. Evidence synthesis

3.1. Study selection and description

Of 599 papers identified in the literature search strategy ([Fig. 1](#)), 294 nonduplicate articles were screened, 34 of which were deemed relevant. A detailed full-text review revealed 15 studies that met the eligibility criteria ([Table 1](#)). Seven studies using whole genome sequencing, shotgun sequencing, or targeted sequencing of the 16S-23S rRNA

region as their NGS methodology were similar and reported enough data to be included in the meta-analysis [5,17,18,20,24,29,30]. Six studies using pathogen-specific PCR were analyzed separately [31–36]. [Supplementary Table 2](#) lists the 19 studies that underwent full-text review but did not meet the inclusion criteria for this systematic review.

In studies comparing NGS with urine culture, the mean age of the included populations ranged between 54 and 85 yr when reported. Urine culture methodology included the use of cystine-lactose-electrolyte-deficient (CLED), blood, or MacConkey agar. NGS platforms included the Ion PGM System (Thermo Fisher, Waltham, MA, USA), Illumina Sequencing Technology (Illumina, San Diego, CA, USA), or Pyromark Q24 (Qiagen, Hilden, Germany). The number of subjects included in each article ranged from 10 to 69 (unreported in one study). Risk of bias was high in each study, and there was significant heterogeneity between studies ($I^2 = 89.92\%$; [Fig. 2](#)).

Of the studies comparing PCR with culture, only one paper reported age statistics; the mean age was 77 yr in that study. Urine culture methodology included the use of CLED, blood, MacConkey, or malt-extract agar. Four studies used quantitative PCR and two used a qualitative PCR approach. PCR protocols varied between studies and were uniquely designed to detect between seven and 25 microbial species. The number of individuals in each study ranged from 81 to 2511. The risk of bias was high for each study.

3.2. Meta-analysis comparing the diagnostic utility of conventional urine culture versus NGS

A meta-analysis was performed for seven similar studies comparing NGS with conventional culture in the identification of bacteria in the urine of patients with suspected UTI [5,17,18,20,24,29,30]. For 274 urine samples from 262 patients, there were significantly more positive NGS tests than there were positive urine cultures ([Fig. 2A](#)). Of the 105 culture-negative samples, 82 were positive for bacteria when NGS testing was used (78.1%). Bacteria identified by conventional urine culture were also identified by NGS testing in 129 of 156 urine samples (82.7%).

We compared species diversity by calculating the difference in H between urine culture and NGS for each study, excluding the study of McDonald et al. [30] who did not enumerate the identified bacterial species. The Shannon diversity index for NGS testing was higher than urine culture in all six studies ([Fig. 2C](#)). Sørensen's coefficient of similarity between culture and NGS results consistently demonstrated divergent results between the diagnostic methods. Aggregate effects of all six studies were computed using a random-effect restricted maximum likelihood model, which demonstrated a significantly higher H for NGS testing than urine culture ($p = 0.0009$). Sørensen's coefficient of similarity between urine culture and NGS testing aggregated across all six studies was found to be 0.31, indicating widely divergent results between methods ([Fig. 2C](#)).

Of a total of 170 different bacterial taxa detected across six studies, 38 (22.4%) were cultivable in standard culture, while 132 (77.6%) were detected by NGS only. Species of 31 genera (18.3%) were detected by both methods and those of seven

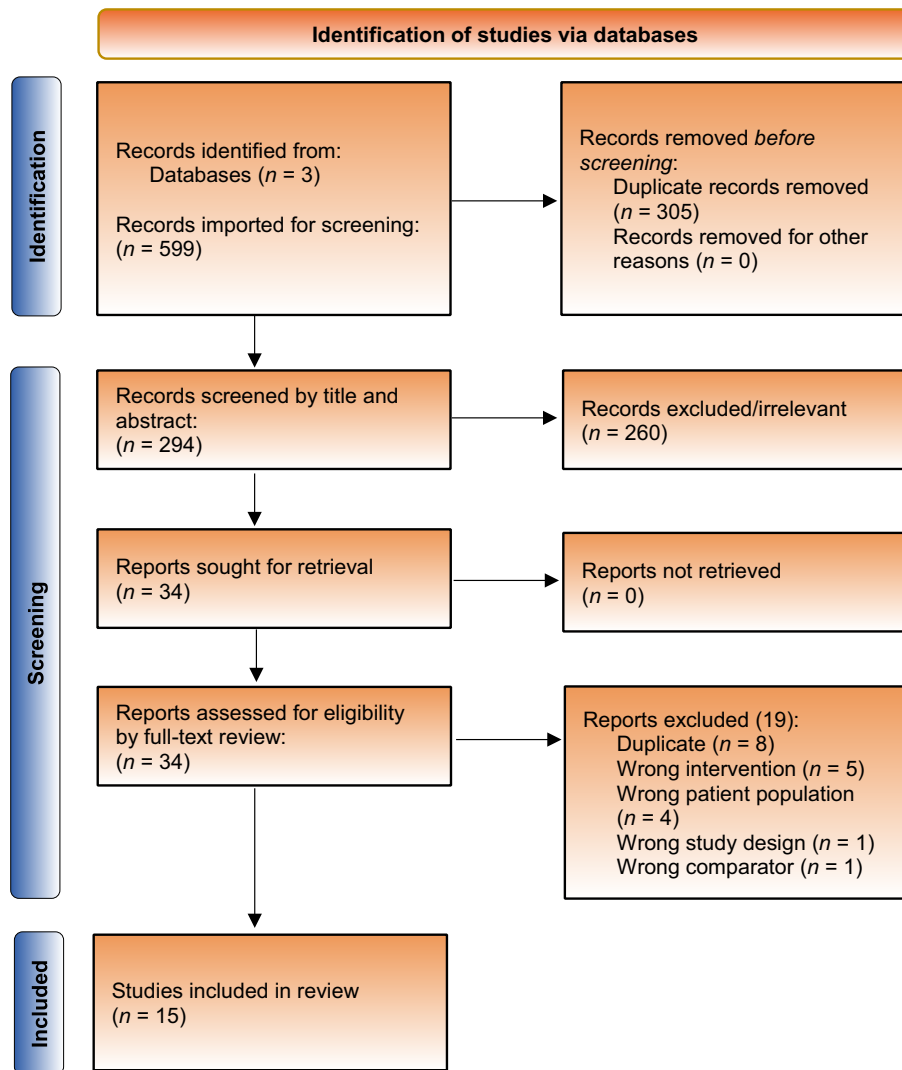


Fig. 1 – PRISMA flow diagram depicting flow of information through the different phases of the systematic review. PRISMA = Preferred Reporting Items for Systematic Review and Meta-analyses.

genera (4.1%) were detected by culture only in specific patients (Supplementary Table 3). Three of the top ten taxa detected by each method (Fig. 3A) were identical between NGS and conventional culture. Overall, NGS detected more obligate anaerobes and fastidious bacteria, whereas urine culture detected predominantly aerobic bacteria.

3.3. Comparison of the diagnostic utility of multiplex PCR using pathogen-specific primers versus conventional urine culture

Six studies comparing PCR with conventional urine culture were included in this systematic review (Table 2) [31–36]. There were substantial differences in PCR methodology and study design; therefore, a meta-analysis could not be performed. In half of the studies [33,35,36], PCR resulted in significantly more positive results than conventional culture, with a greater percentage of PCR-positive/culture-negative urine samples relative to PCR-negative/culture-positive urine samples. In contrast, the remaining three

studies [31,32,34] failed to detect a significant difference between PCR and culture. Of papers reporting the microbial taxa identified with each method, species diversity was significantly greater with PCR than with culture in two studies [32,33], but not significantly different in two others (Table 2) [31,34]. Comparison of the top taxa detected by PCR and conventional culture (Fig. 3B) revealed that nine of the top ten taxa were identical between both methods.

3.4. Comparison of resistome profiles determined by conventional urine culture versus molecular diagnostic methods

Seven studies [17,18,29,30,33,36,37] examined the resistome profiles determined by conventional urine culture (phenotypic antibiotic susceptibility) and molecular diagnostic (genotypic antibiotic susceptibility) testing, but only four studies [17,18,33,37] reported the results of both.

Of the two NGS studies [17,18] reporting phenotypic and genotypic antibiotic susceptibility, antibiotic susceptibility testing was performed on 52 bacterial isolates. Antibiotic

Table 1 – Summary of comparative studies included in the analysis

Reference, study design, country	Total number of participants	Risk of bias	Population description	Inclusion criteria	Exclusion criteria	Conventional culture method used	Molecular method used	Resistome testing	Patient symptoms' response to antibiotics
<i>Next-generation sequencing</i>									
Barraud et al. [17], prospective, France	40	High	Adult patients, mean age 71 yr (SD ± 17 yr); 23 female (56%); 17 male (44%)	UTI-related sepsis	Antibiotic use in the last 10 d	CLED agar plates, incubation 16–48 h; bacterial count determined from 10 ² to 10 ⁶ cfu/ml; bacterial ID with VITEK system	NGS, Ion Proton system	Yes	No
Hasman et al. [18], comparative retrospective, Denmark	35	High	Adults patients	Suspected UTI	Not stated	Blood agar plates with no dilution, 10-fold, and 100-fold dilution	NGS, Ion Torrent PGM system	Yes	No
Sabat et al. [20], comparative retrospective, the Netherlands	60	High	Adult patients	Suspected UTI	Not stated	Stated as routine diagnostics by the Department of Bacteriology at Certe	NGS, Illumina MiSeq sequencing	No	No
Burnham et al. [29], retrospective comparative, USA	31	High	Adult patients	Developed bacteria UTI within the first 12 mo of transplantation	Not stated	Tryptic soy agar with sheep blood and MacConkey agar, incubated in ambient air at 35 °C	NGS, Illumina NextSeq system	Yes	No
Ishihara et al. [24], comparative retrospective, Japan	10	High	Adult patients in the ED; median age 85 yr; 3 male (30%), 7 female (70%); 4/10 with sepsis, 3/10 with septic shock	Suspected acute UTI (upper and lower)	Not stated	Not stated	NGS, Ion PGM system	No	No
Yoo et al. [5], comparative retrospective, Korea	42	High	Adult patients visiting a tertiary care hospital; mean age 54 yr (SD ± 12 yr); 100% female; 24/42 postmenopausal (64.2%); 23/42 had prior antibiotic use (54.7%)	Suspected acute uncomplicated cystitis or recurrent cystitis	Anatomical or structural abnormalities; nephrolithiasis; pregnancy; prolonged indwelling catheter	Blood agar plate and MacConkey agar plate, incubated under standard conditions; bacteria count >10 ³ cfu/ml	NGS, Illumina MiSeq sequencing	No	No
McDonald et al. [30], RCT, USA	44 (+22 controls)	High	Adult patients; 15/44 male (34%), 29/44 female (66%); 19/44 with complicated UTI (43%)	Suspected uncomplicated and complicated UTI (defined as men or indwelling urinary catheter)	Fever, clinical symptoms of acute pyelonephritis, acute or chronic prostatitis, urethritis, and epididymitis, treated for UTI in the past month	Stated as standard protocol as per Florida Hospital pathology laboratory with antibiotic susceptibility testing if common uropathogens are present in >10 ⁵ cfu/ml.	NGS, Illumina MiSeq system (MicrogenDx); resistance factor assay; semiquantitative assay	Yes	Yes
Rajagopalan et al. [40], study type unclear, USA	Not stated	Indeterminate	Adult patients; age ≥75 yr, nursing home residents	Suspected UTI	Not stated	Not stated	NGS, PyroMark Q24 system	No	No
Coba et al. [37], prospective, USA	69	Indeterminate	Adult patients	Chronic UTI with symptom flare	Not stated	Not stated	NGS, Illumina MiSeq system (MicrogenDx)	Yes	No

(continued on next page)

Table 1 (continued)

Reference, study design, country	Total number of participants	Risk of bias	Population description	Inclusion criteria	Exclusion criteria	Conventional culture method used	Molecular method used	Resistome testing	Patient symptoms' response to antibiotics
<i>Polymerase chain reaction</i>									
Wojno et al. [36], comparative retrospective, USA	582	High	Adult patients; mean age 77 years (SD ± 8 yr); 347 were male (60%), 235 female (40%); 89 (15.3%) with antibiotic usage in last 3 wk	Age >60 yr; suspected acute cystitis or UTI	Age <60 yr	Blood agar plates, plates with half colistin and nalidixic acid agar and half MacConkey agar; incubated as per routine protocol; plates with <10 ⁴ cfu/ml were reported as normal urogenital flora	Bacterial pathogen-directed qPCR amplification of 25 uropathogens (Pathnostics); samples spotted on 112-format OpenArray chips	Yes	No
Lehmann et al. [31], comparative prospective, Germany	189	High	Adult patients; 100 ICU patients; 89 outpatients, 96 female (50.8%), 93 male (49.2%); 40.2% lower UTI, 59.8% complicated UTI	Suspected UTI	Enterovesical fistulae or gut segments autotransplanted into the urinary tract	CLED, MacConkey, and malt-extract agar, cultured 18–24 h at 36 °C	Bacterial pathogen-directed qualitative PCR amplification of 16S rRNA region of 15 uropathogens	No	No
Lehmann et al. [32], comparative prospective, Germany	81	High	Adult patients; 43 female (53%), 38 male (47%); 48% lower UTI, 52% complicated UTI	Suspected UTI	Enterovesical fistulae or gut segments autotransplanted into the urinary tract	CLED, MacConkey, and malt-extract agar, cultured 24 h at 36 °C; defined infection if ≥10 ⁵ cfu/ml reported	Bacterial and fungal pathogen-directed qualitative PCR amplification of ITS region of 20 uropathogens	No	No
Sun et al. [33], comparative prospective, China	531	High	Adult patients (in- and outpatient)	Suspected UTI	Not stated	Columbia blood agar cultured 24 h at 37 °C; defined infection if ≥10 ⁴ cfu/ml reported.	Bacterial pathogen-directed qPCR amplification of conserved genomic fragments of 18 uropathogens	Yes	No
Van der Zee et al. [34], comparative prospective, the Netherlands	211	High	Adult patients (in- and outpatient)	Suspected UTI	Not stated	Blood agar and MacConkey agar; defined infection if ≥10 ³ cfu/ml reported	Bacterial pathogen-directed semiquantitative PCR amplification of 16S rRNA regions of 7 uropathogens	No	No
Vollstedt et al. [35], comparative prospective, USA	2511	High	Adult patients	>60 yr old, suspected UTI	>60 yr old	Not stated	Pathnostics qPCR (details not stated)	No	No

cfu = colony-forming units; CLED = cysteine-lactose-electrolyte deficient; ED = emergency department; ICU = intensive care unit; ID = identification; NGS = next-generation sequencing; PCR = polymerase chain reaction; qPCR = quantitative polymerase chain reaction; RCT = randomized controlled trial; SD = standard deviation; UTI = urinary tract infection.

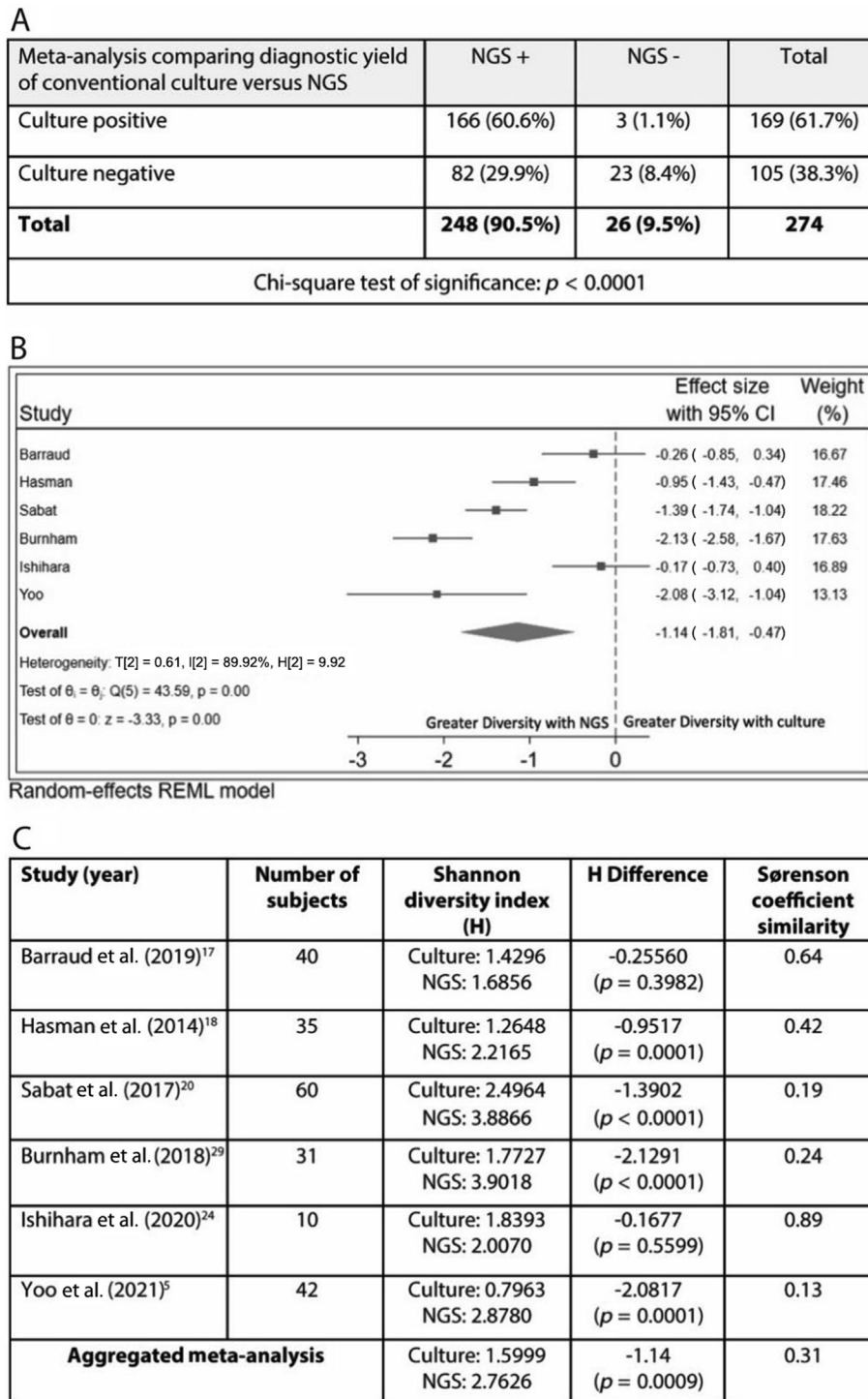


Fig. 2 – Meta-analyses of species diversity and heterogeneity. (A) A meta-analysis comparing the number of positive and negative results of conventional urine culture versus NGS. **(B)** A meta-analysis forest plot of the effect size (difference in diversity H between urine culture and NGS) and calculation of heterogeneity. **(C)** The Shannon Diversity Index (H) and Sørensen's coefficient of similarity between conventional urine culture and NGS were calculated individually for each study as well as in an aggregate analysis of all six studies. CI = confidence interval; NGS = next-generation sequencing; REML = restricted maximum likelihood.

susceptibility testing was concordant between both methods in 44 out of 52 samples (84.6%) and was discordant in eight out of 52 samples (15.4%; Table 3). Coba et al. [37] reported in their abstract that in 69 individuals, antibiotic resistance was concordant between methods in 15 (21.7%), partially

concordant in ten (14.5%), and discordant in 44 (63.8%; $p = 0.0001$). In the single PCR study [33] reporting phenotypic and genotypic antibiotic susceptibility, 36 drug-resistant phenotypes were identified by culture, including extended-spectrum beta lactamase (ESBL) *E. coli*, carbapenem-

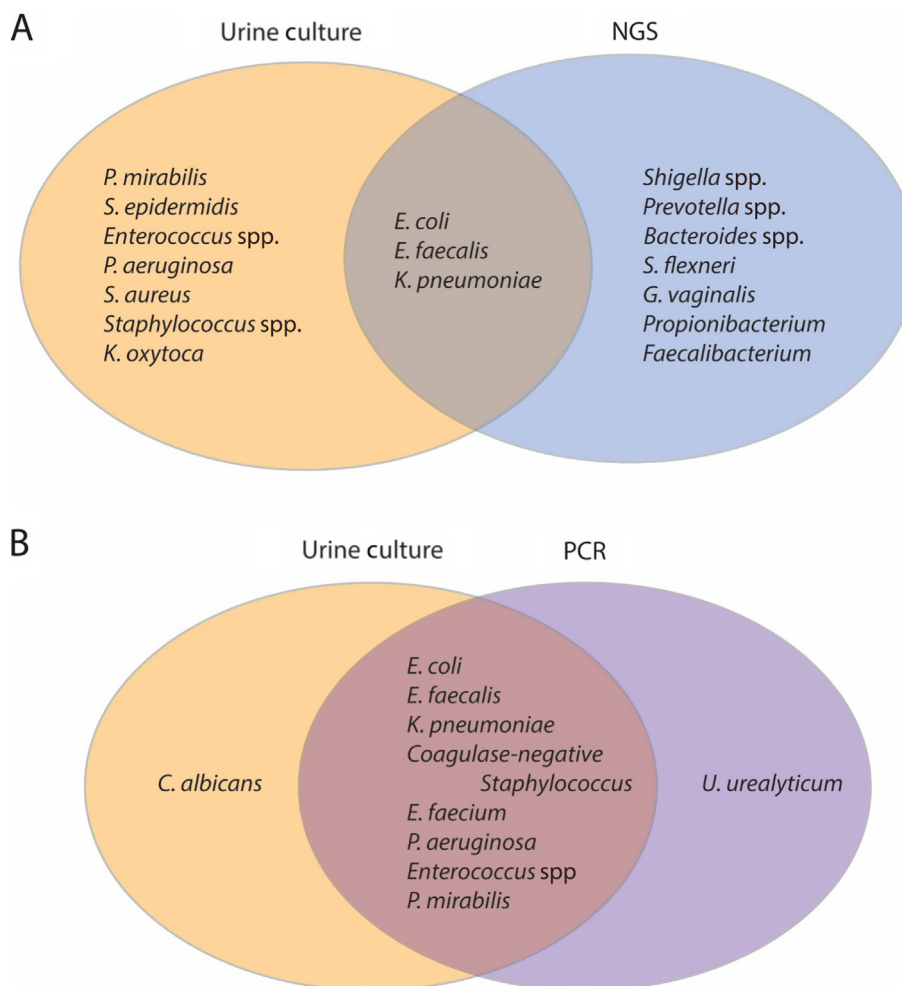


Fig. 3 – Comparison of the top ten most common bacteria detected by conventional culture and molecular diagnostic methods: (A) NGS and (B) PCR. Only the top ten bacteria detected by each form of testing are included; this figure does not reflect the depth of all detected taxa. This figure does not imply that NGS missed many of the traditional uropathogens identified by culture, but only demonstrates the increased sensitivity in detecting bacteria not detected by culture. In contrast, PCR selects the taxa of interest, the majority of which were selected based on prior data from urine culture, leading to a greater overlap of detection. NGS = next-generation sequencing; PCR = polymerase chain reaction.

resistant *Pseudomonas aeruginosa*, carbapenem-resistant *Klebsiella pneumoniae*, and ESBL *Proteus mirabilis*. In contrast, drug-resistant genes were detected only in 31 samples, with more than one drug-resistant gene recognized in nine samples. Of the nine samples in which multiple resistance genes were detected, at least one matched the phenotypic resistance profile for each sample. Overall, the data demonstrated that 86.97% of urine samples with a positive drug-resistant phenotype exhibited the corresponding drug-resistant gene type.

In the follow-up study to Wojno et al. [36] by Baunoch et al. [38], the resistomes of 1155 patient urine samples were analyzed using multiplex PCR and pooled antibiotic susceptibility testing. The authors found 60% concordance between the presence of antibiotic-resistant genes and corresponding antimicrobial susceptibility. Aminopenicillins, beta-lactamase inhibitor combinations, fluoroquinolones, and carbapenems had concordance rates of >67.2%, while cephalosporins and glycopeptides had lower concordance rates of 48.5% and 56.2%, respectively [38].

3.5. Comparison of patient symptom response with antibiotic selection guided by conventional urine culture versus NGS

Only one study [30] compared outcomes for 44 patients with symptoms of acute cystitis whose antibiotic selection was guided by conventional urine culture or NGS. All 44 individuals had positive NGS tests, while only 13 had positive urine cultures. Symptom severity at testing was compared with symptoms 14 d later using the UTI Symptoms Assessment (UTISA) questionnaire. Each of the 22 patients were randomly allocated to treatment based on urine culture or NGS testing. Seven patients who had a positive urine culture were treated on day 1 based on the phenotypic antibiotic susceptibility testing, whereas 15 with negative urine culture were treated on day 8 based on NGS results. Of those initially treated based on NGS testing, urine culture was positive in six and negative in 16. UTISA scores were not statistically different between both groups upon entry into the study (9.00 vs 10.22). UTISA scores (possible scores 0–21, with 21 indicating more severe symptoms) demon-

Table 2 – Summary of results of individual studies comparing polymerase chain reaction (PCR) with conventional urine culture in the diagnosis of urinary tract infection (UTI)

Study (year)	Number of urine samples	Number culture positive	Number PCR positive	Chi-square <i>p</i> value	Number of PCR positive, culture negative	Number of PCR negative, culture positive	Number of species detected with culture	Number of species detected with PCR	Shannon Diversity Index (H)	Concordance between PCR and culture when result positive	Sørensen's coefficient of similarity
Wojno et al. (2020) [36]	582	217 (37.3%)	326 (56%)	<0.001	130/365 (36%)	21/256 (8.2%)	22	25	Unknown	90.3%	0.68
Lehmann et al. (2010) [31]	301	83 (27.6%)	81 (26.9%)	0.995	15/218 (6.8%)	17/220 (7.7%)	14	10	Culture: 2.00 PCR: 1.74 (<i>p</i> = 0.06)	75.2%	0.83
Lehmann et al. (2011) [32]	82	67 (81.7%)	61 (74.4%)	0.402	6/15 (40%)	12/21 (57.1%)	10	13	Culture: 1.71 PCR: 2.02 (<i>p</i> = 0.03)	90.5%	0.82
Sun et al. (2021) [33]	531	291 (54.8%)	334 (62.9%)	0.003	43/240 (17.9%)	0/197 (0%)	13	13	Culture: 2.17 PCR: 2.39 (<i>p</i> < 0.001)	100%	0.84
Van der Zee et al. (2016) [34]	211	54 ^a (25.6%)	62 ^a (29.4%)	0.186 ^a	18/104 ^a (17.3%)	10/96 ^a (10.4%)	10	7	Culture: 1.58 PCR: 1.47 (<i>p</i> = 0.52)	82%	0.67
Vollstedt et al. (2020) [35]	2511	1098 (43.7%)	1575 (62.7%)	<0.001	557/1413 (39.4%)	3/936 (3.2%)	Unknown	Unknown	Unknown	Unknown	Unknown

^a Fifty-three inconclusive tests were left out of analysis.

Table 3 – Discordant phenotypic susceptibility testing and genotypic resistome testing results

Study ID	Study urine sample number	Phenotypic susceptibility testing resistance	Genotypic resistome testing
<i>Next-generation sequencing</i>			
Barraud et al. [17]	P008	Nalidixic acid	None detected
Barraud et al. [17]	T039	Amoxicillin, ticarcillin	None detected
Barraud et al. [17]	T048	Amoxicillin, ticarcillin	None detected
Hasman et al. [18]	10	None detected	Extended-spectrum cephalosporinase
Hasman et al. [18]	21	Ampicillin, ciprofloxacin, gentamicin, nalidixic acid	Ampicillin, gentamycin, tetracycline
Hasman et al. [18]	27	None detected	Tetracycline
Hasman et al. [18]	28	Penicillin, sulfamethoxazole	Penicillin
Hasman et al. [18]	34	Tetracycline	Tetracycline, streptomycin
<i>Polymerase chain reaction</i>			
Sun et al. [33]	Unknown	Extended-spectrum beta-lactamase	None detected (4)
Sun et al. [33]	Unknown	Carbapenemase	None detected (2)

strated significantly greater symptomatic improvements in those treated based on NGS testing compared with those treated based on urine culture (average net improvement of 8.5 vs 3.7, $p < 0.001$). Individuals with negative urine culture who were treated on day 8 based on NGS results were found to have an average UTISA score improvement of 7.4, which the authors state is a substantial improvement compared with the group treated based on urine culture alone.

4. Conclusions

The results of this systematic review and meta-analysis comparing conventional urine culture with molecular diagnostic methods in the diagnosis and treatment of UTI allow the following conclusions:

1. NGS is significantly more sensitive than conventional urine culture in the identification of bacteria in the urine of adults with symptoms of acute UTI (quality of evidence: moderate).
2. NGS can identify the same uropathogenic organism as conventional urine culture in most cases; however, these diagnostic tests have widely divergent results due to the very high sensitivity of NGS (quality of evidence: moderate).
3. Species diversity detected using NGS is greater than that using urine culture (quality of evidence: moderate).
4. The genotypic resistome detected by molecular methods is similar to the phenotypic resistome detected by standard susceptibility testing in most, but not all, cases (quality of evidence: low).
5. Conclusions comparing PCR with conventional culture depend largely upon PCR design in the selection of microbial pathogens. PCR protocols designed to detect a larger number and more diverse range of microbes had increased sensitivity and species diversity compared with urine culture (quality of evidence: moderate).
6. Conclusions regarding patient symptom response to antibiotic therapy guided by any molecular test versus standard susceptibility testing could not be made, given that only a single study with a high risk of bias sought to answer this question.

The application of molecular-based microbial profiling to the diagnosis of UTI has implicated a wider range of pathogenic microorganisms in patients with cystitis-like symptoms; it has also exposed important shortcomings that limit

the interpretability of results. For example, molecular-based methods are highly sensitive in the detection of bacterial and fungal species, making it difficult to discern commensal from pathogenic micro-organisms, which might represent contaminants from the vagina, urethra, urethral meatus, or perineal skin. In contrast to conventional urine culture that relies on the growth of live bacteria for species identification, NGS and PCR alone cannot distinguish actively expanding pathogens from either dead or quiescent microbes. Additionally, NGS and PCR can test only for genotypic antimicrobial susceptibility rather than phenotypic susceptibility, which is not always concordant; as yet, no study has compared the respective utilities to detect antimicrobial resistance in disease management. Taken together, these limitations leave many questions unanswered regarding the clinical interpretability of results. Finally, other limitations of NGS include reproducibility of results, given variable findings obtained by different protocols and laboratories; quality of data in reference libraries that currently include poorly annotated sequences, duplications, missing genera, and missing species; and lack of standardized algorithms to predict the causative pathogen [20,22,26,39].

It should be noted that only one study included in this review used control individuals without symptoms of UTI in the study design and analysis [30]. Of 22 asymptomatic controls, 21 (95%) had positive NGS tests versus five (23%) who had positive urine cultures. Although the study did not address whether there were differences in the bacterial composition profiles between experimental patients and controls, this finding underscores an important limitation of molecular diagnostic approaches in the management of UTI: although highly sensitive, NGS has poor specificity.

While the results of McDonald et al. [30] suggest potential utility of NGS testing in an appropriate population with a high suspicion of UTI, limited data on the outcomes of treatment guided by these results make it challenging to identify the specific population that would benefit from molecular testing. Considering positivity rates in asymptomatic patients of 95%, treatment of all bacteria detected by molecular testing in an unselected population with non-specific urinary complaints is likely to result in both incorrect diagnoses and significant overtreatment with antimicrobial therapies. Thus, strict adherence to antibiotic stewardship guidelines is essential to minimizing antibiotics misuse. Additionally, a better understanding of which bacteria (species and strain) constitute uropathogens and would benefit from antimicrobial therapy may help define

the utility of this highly sensitive testing in clinical practice. As of yet, NGS and PCR do not have the same capacity as cultivation of live urinary isolates to permit the study of microbial virulence and pathogenicity in determining the species responsible for infection.

There are several limitations of this systematic review. The number of studies included in the systematic review is small, and they all carry a high risk of bias. Corporate sponsorship was present in five studies; Pathnostics (Irvine, CA, USA) sponsored three studies [35,36,38], while MicroGenDx (Lubbock, TX, USA) provided support for two studies [30,37]. Additionally, significant heterogeneity exists between studies owing to the different characteristics of included participants and different protocols of the molecular diagnostic methods used.

Finally, while molecular diagnostic technology has the potential to revolutionize clinical management of UTI, additional data are needed to address how molecular methods should guide patient treatment and symptom response. This review should serve as a call to action for the development of well-designed studies to evaluate (1) efficacy of antibiotic selection using molecular methods versus conventional urine culture, (2) patient symptomatic responses following treatment based on molecular methods in comparison with urine culture, (3) patient population(s) in which molecular diagnostics should be used, and (4) short- and long-term changes in antibiotic resistance profiles of bacteria in urine following treatment guided by molecular diagnostics in contrast to urine culture.

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Study concept and design: Szlachta-McGinn, Ackerman.

Acquisition of data: Szlachta-McGinn, Douglass, Ackerman.

Analysis and interpretation of data: Szlachta-McGinn, Douglass, Ackerman, Chung, Jackson.

Drafting of the manuscript: Szlachta-McGinn, Douglass, Ackerman.

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Appendix A. Supplementary data

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