One Health Microbiology & Infection

OPEN ACCESS

eISSN:3054-5161

Research article

Multiplex PCR for detection of bacterial uropathogens in nosocomial samples from northeastern Mexico: One Health relevant urinary tract pathogens

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Article history: Received 28 June 2026; Accepted 12 August 2026; Available online 19 August 2026
Article information: Saenz-Santos CM et al., 2026. *One Health Microbiol. Infect*. Vol. 2, No. 1: 144-151. https://doi.org/10.66585/ohmi.2026.2.0025

Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, accounting for approximately 150 million cases annually, including an estimated 3.8 million in Mexico. The principal uropathogens are *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, all of which are opportunistic zoonotic bacteria associated with humans, companion animals, livestock, and wildlife. Within the One Health framework, these pathogens and their antimicrobial resistance (AMR) determinants may circulate across healthcare settings, communities, animals, and the environment, highlighting the need for integrated surveillance. Although conventional urine culture remains the diagnostic gold standard, rapid molecular methods can facilitate timely diagnosis and support antimicrobial stewardship. This study analyzed 300 urine samples collected from three hospitals representing different levels of healthcare complexity in Reynosa, Tamaulipas, Mexico. Conventional urine culture and species-specific PCR assays targeting *E. coli*, *K. pneumoniae*, and *P. mirabilis* were performed. Fifty-six samples yielded positive urine cultures, with *E. coli* identified as the predominant uropathogen (85.7%, 48/56), followed by *K. pneumoniae* (10.7%, 6/56) and *P. mirabilis* (3.6%, 2/56). Positive cases were more frequent among females (70%). In addition, a multiplex PCR assay was developed and validated to simultaneously detect the three pathogens. The assay was also successfully applied directly to unprocessed urine samples, provided that urine represented no more than 20% of the total PCR reaction volume. The multiplex PCR demonstrated 100% concordance with conventional culture results while substantially reducing diagnostic turnaround time. To our knowledge, this is the first study conducted in Reynosa, Tamaulipas, to integrate conventional microbiological and molecular diagnostics for UTIs caused by One Health bacterial pathogens. These findings establish a methodological baseline for regional surveillance of uropathogens at the human–healthcare–environment interface, supporting future cross-border One Health surveillance initiatives.

Keywords: Mexico-USA border region, Multiplex PCR, Nosocomial surveillance, One Health approach, Uropathogens

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in both community and hospital settings. Significant bacteriuria, defined as ≥ 10^5 colony-forming units (CFU)/mL of urine, remains the conventional diagnostic threshold for clinically relevant infection [1, 2]. *Escherichia coli* (*E. coli*) is the predominant uropathogen, followed by *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus mirabilis* (*P. mirabilis*), and other Enterobacterales [1]. Beyond their clinical importance, uropathogens represent an increasing concern from a One Health perspective. An important but often overlooked aspect of urinary tract infections from a One Health perspective is that uropathogens are continuously shed in the urine of infected or colonized individuals. Following excretion, these microorganisms may enter municipal wastewater systems or, in areas with inadequate sanitation or untreated sewage discharge, be released directly into surface waters and surrounding environments. In wastewater, *E. coli*, *K. pneumoniae*, and *P. mirabilis* can persist and disseminate, creating opportunities for exposure of domestic animals, livestock, wildlife, and humans through contaminated water or environmental reservoirs. In the Reynosa-McAllen border region, recurrent flooding, wastewater discharges into the Rio Grande (Río Bravo), agricultural water reuse, and intense human and animal interactions may facilitate the environmental dissemination and recirculation of these uropathogens. These factors underscore the importance of investigating One Health-relevant urinary tract pathogens that integrate human, animal, and environmental health surveillance [3, 4, 5].

The Reynosa-McAllen metropolitan region on the United States-Mexico border represents a unique epidemiological setting due to intense cross-border population movement, differences in antimicrobial prescribing practices and healthcare systems, and continuous exposure to agricultural and environmental sources of AMR. Characterizing the molecular epidemiology of uropathogens in this region is therefore relevant not only for improving patient management but also for strengthening integrated surveillance within a One Health framework [6, 7].

Although conventional urine culture remains the reference standard for UTI diagnosis [8], it typically requires 18-48 h to generate results. It may yield false-negative results in patients who have received prior antimicrobial therapy. Rapid molecular diagnostics have

emerged as valuable complementary tools to conventional microbiological methods [9]. Multiplex polymerase chain reaction (mPCR) enables simultaneous detection of multiple pathogens within a few hours, substantially reducing diagnostic turnaround time. Yet most PCR protocols require prior DNA extraction to remove urinary inhibitors, particularly urea, which increases laboratory time, complexity, and cost [8].

Based on previous studies demonstrating successful direct PCR amplification from complex biological matrices [10], we hypothesized that unprocessed urine could be used directly as the PCR template, up to a defined volume threshold, without compromising amplification efficiency. We further hypothesized that the initial denaturation step would provide sufficient lysis of bacterial cells to release genomic DNA, eliminating the need for DNA purification. Accordingly, the objectives of this study were to develop and validate a rapid, simple, and cost-effective multiplex PCR assay for the simultaneous detection of *E. coli*, *K. pneumoniae*, and *P. mirabilis* directly from urine samples, and to evaluate its agreement with conventional urine culture. By integrating rapid molecular diagnostics with conventional microbiology, this work provides a practical tool to support clinical diagnosis and regional surveillance of uropathogens and their distribution within a One Health framework.

Materials and methods

Study design and setting

A cross-sectional observational study was conducted from July to December 2025 at three healthcare institutions in Reynosa, Tamaulipas, Mexico, representing different levels of medical care: Pemex Regional Hospital (PRH), a secondary-care occupational hospital; Santander Hospital (SH), a private hospital; and José María Cantú Garza General Hospital (JMCGH), a public tertiary-care hospital. Including institutions serving distinct patient populations enabled evaluation of uropathogen distribution across healthcare settings within a One Health surveillance framework.

Participants and sample collection

A total of 300 urine samples were collected from patients presenting with clinical signs and symptoms suggestive of urinary tract infection (UTI), including dysuria, urinary frequency, urgency, suprapubic discomfort, or flank pain. Inclusion criteria included patients with clinical suspicion of UTI who provided a clean-catch midstream urine specimen. Patients receiving antimicrobial therapy within 48 h before urine collection were excluded. Recorded demographic information included age, sex, and hospital of origin. Urine specimens were collected in sterile wide-mouth containers, transported to the laboratory under refrigeration (2–8°C) within 2 h of collection, and processed immediately or stored at –20°C until analysis.

Conventional urine culture

Samples were homogenized according to CLSI 2024 guidelines [11]. A calibrated 1-µL loop was used to inoculate each sample onto Cystine Lactose Electrolyte Deficient (CLED) agar (BD Bioxon™, Becton Dickinson, Mexico), which was incubated aerobically at 35 ± 2°C for 18–24 h. Cultures yielding ≥ 10^5 CFU/mL of a single or predominant organism were considered positive. Samples with polymicrobial growth (≥ 3 morphotypes) were classified as contaminated and excluded. Bacterial identification was performed using the Beckman Coulter MicroScan Gram-Negative Panel system (Siemens Healthcare Diagnostics, Benito Juarez, Ciudad de México), with identification based on biochemical reactions.

DNA extraction

Total DNA was extracted from all urine samples using the phenol–chloroform–isoamyl alcohol (25:24:1) method, following the protocol described by Green and Sambrook [12]. The original protocol was modified only to adapt it for urine specimens. Briefly, 5 mL of urine was centrifuged at 10,000 rpm for 5 min to concentrate bacterial cells. The resulting pellet, rather than the 500 µL of whole blood specified in the original protocol, was then processed. DNA concentration and purity were measured using a Nanodrop 1000 spectrophotometer, and integrity was verified by 1% agarose gel electrophoresis with ethidium bromide staining. Samples with OD260/280 ratios between 1.7 and 2.0 were considered suitable for PCR analysis.

Primer design

Species-specific primers targeting the *ual* gene of *E. coli* (GenBank accession no. CP000244.1), the *ntrA* gene of *K. pneumoniae* (GenBank accession no. CP003223.1), and the *ureR* gene of *P. mirabilis* (GenBank accession no. CP098448.1) were designed to generate amplicons of 147, 120, and 225 bp, respectively. Amplicon sizes were selected to allow simultaneous resolution by agarose gel electrophoresis. Primer sequences are shown in Table 1. Primer sequences were analyzed *in silico* using Primer-BLAST against the NCBI RefSeq bacterial genome database, and no off-target amplicons meeting the selected search criteria were detected.

PCR assay optimization

PCR reactions (25 µL) contained template DNA, 25 pmol of each primer, 5 µL of 5× MyTaq reaction buffer, and 1 U of MyTaq DNA polymerase. Cycling conditions consisted of 94°C for 2 min; 35 cycles of 94°C for 10 sec, 50°C for 30 sec, and 72°C for 30 sec; followed by a final extension at

72°C for 5 min. Products were resolved on 2% agarose gels stained with RedSafe. Negative controls were included in all assays, and primer specificity was evaluated using reference strains (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. mirabilis* ATCC 7002).

Table 1: Primers, target genes, and amplification sizes of the genes used for the detection of *E. coli*, *K. pneumoniae*, and *P. mirabilis*.

Pathogen	Sequence	*Gen*
E. coli,	5′-AAAACGGCAAGAAAAAGCAG-3′ 5′-ACGCGTGGTTACAGTCTTGCG-3′	*Ual*, 147 bp
K. pneumoniae	5′-CATCTCGATCTGCTGGCCAA-3′ 5′-GCGCGGATCCAGCGATTGGA-3′	*ntrA*, 120 bp
P. mirabilis	5′-GGTGAGATTTGTATTAATGG-3′ 5′-ATAATCTGGAAGATGACGAG-3′	*ureR*, 225 bp

Determination of the maximum non-inhibitory urine volume

Fresh, culture-negative urine spiked with purified bacterial DNA was used to determine the maximum urine volume compatible with PCR amplification. Urine volumes of 1-6 µL were tested in triplicate in 25 µL reactions. The experiment was independently repeated for each target species.

Direct PCR from unprocessed urine

To evaluate direct amplification without DNA extraction, fresh urine was inoculated with approximately 400–500 bacterial cells/µL. An aliquot of the inoculated urine (5 µL) was used directly as the PCR template, relying on thermal lysis during the initial denaturation step. Experiments were performed independently for each bacterial species.

Multiplex PCR standardization

Duplex PCR (*E. coli*/*P. mirabilis*) and triplex PCR (*E. coli*/*K. pneumoniae*/*P. mirabilis*) assays were optimized by combining the corresponding primer sets in a single reaction. The multiplex PCR assays were performed using the same reaction mixture, reagent concentrations, and amplification conditions as the conventional PCR assays. The only modification was adjusting the primer mix volume to match the multiplex design, and the corresponding volume was subtracted from nuclease-free water to maintain a constant final reaction volume.

Statistical analysis

Positivity rates by hospital and sex were compared using the chi-square (χ^2) test. Statistical significance was set at $p < 0.05$. 95% confidence intervals were calculated using the Wilson score method. Statistical analyses were conducted in IBM SPSS software V.25.

Results

Urine culture positivity and demographic distribution

A total of 300 urine samples were collected from patients presenting with clinical signs suggestive of urinary tract infection at three healthcare institutions. Overall, 56 samples (18.7%) yielded positive urine cultures, whereas 244 (81.3%) were culture-negative. Positivity rates differed significantly among institutions ($\chi^2 = 23.28$, df = 2, $p < 0.001$). PRH had the lowest positivity rate (13.1%, 32/245), followed by SH (37.5%, 9/24), while JMCGH had the highest rate (48.4%, 15/31) (Table 2). Among culture-positive patients, females accounted for 69.6% (39/56) and males for 30.4% (17/56). This distribution was similar across the three participating hospitals (Table 3).

Table 2: Number and rate of culture-positive urine samples by hospital.

Hospital	Total samples	Positive	Negative	Positivity rate (%)
PRH	245	32	213	13.1
SH	24	9	15	37.5
JMCGH	31	15	16	48.4

PRH = PEMEX Regional Hospital, SH = Santander Hospital, JMCGH = José María Cantú Garza General Hospital.

Table 3: Number and rate of culture-positive urine samples by hospital and sex.

Hospital	Female	Male	Total positive cases
PRH	23 (71.9%)	9 (28.1%)	32
SH	6 (66.7%)	3 (33.3%)	9
JMCGH	10 (66.7%)	5 (33.3%)	15

PRH = PEMEX Regional Hospital, SH = Santander Hospital, JMCGH = José María Cantú Garza General Hospital.

Bacterial identification by conventional urine culture

Among the 56 positive urine cultures, *E. coli* was the predominant uropathogen, accounting for 85.7% (48/56) of isolates. *K. pneumoniae* accounted for 10.7% (6/56), and *P. mirabilis* was identified in 3.6% (2/56) of positive cultures (Figure 1).

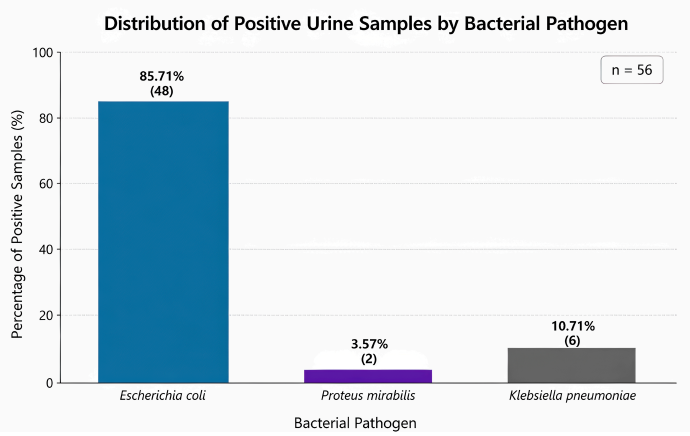

Figure 1: Distribution of positive urine samples by bacterial pathogen determined by conventional urine culture. *E. coli* was the predominant uropathogen, accounting for 85.71% of positive cultures, followed by *K. pneumoniae* (10.71%) and *P. mirabilis* (3.57%). The positivity rate was 18.67% among the analyzed samples.

Quality assessment of DNA extraction

Genomic DNA was successfully extracted from all 300 urine samples, and DNA integrity was confirmed by 1% agarose gel electrophoresis with ethidium bromide staining, revealing intact high-molecular-weight DNA (Figure 2). DNA concentration and purity were measured using a Nanodrop 1000 spectrophotometer. Representative DNA samples yielded concentrations of approximately 42.6 ng/µL and an OD260/280 ratio of 1.8, indicating adequate purity for PCR amplification. All samples included in subsequent analyses exhibited OD260/280 ratios ranging from 1.7 to 2.0.

Analytical sensitivity of the PCR assay

The analytical sensitivity of the PCR assay was evaluated using ten-fold serial dilutions of purified *E. coli* genomic DNA, ranging from 42.6 ng to 0.04 pg. Amplification of the expected 147-bp *ual* amplicon was detected across the entire dilution series (Figure 3), demonstrating that the assay remained capable of detecting the lowest DNA concentration tested.

Conventional PCR using purified genomic DNA

Species-specific PCR was performed on genomic DNA extracted from all 300 urine samples. Amplification products of the expected sizes were obtained for each target organism: 147 bp for *E. coli* (*ual*), 120 bp for *K. pneumoniae* (*ntrA*), and 225 bp for *P. mirabilis* (*ureR*) (Figure 4A). PCR results were fully concordant with conventional urine culture, with amplification detected exclusively in samples positive for the corresponding bacterial species.

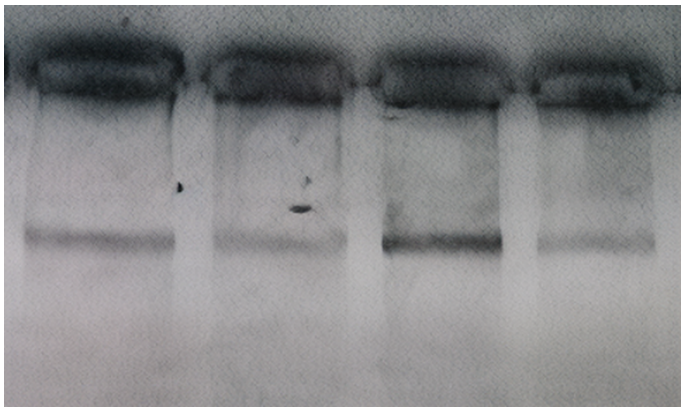

Figure 2: Representative gel of DNA extraction. DNA is observed in all four lanes, confirming the effectiveness of the phenol–chloroform extraction method (1% agarose gel stained with ethidium bromide and visualized under a UV transilluminator).

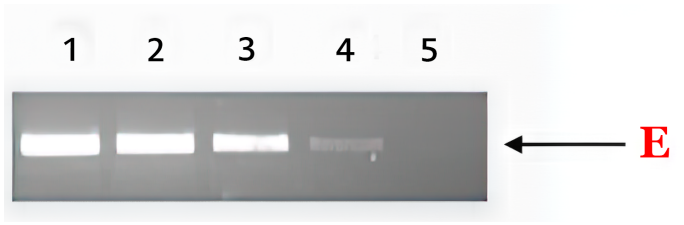

Figure 3: Representative gel showing the detection level of DNA extracted from urine samples. The first lane shows the 42 ng dilution; the second lane, the 4.2 ng dilution; the third lane, 0.42 pg; the fourth lane, the 0.04 pg dilution; and the fifth lane, the negative control. The amplified product of *E. coli* (E) corresponds to 147 bp.1% agarose gel stained with 1.6 µL RedSafe.

Determination of the maximum non-inhibitory urine volume

To evaluate the inhibitory effect of urine on PCR amplification, increasing volumes (1-6 µL) of culture-negative urine spiked with purified *E. coli* DNA were added to 25 µL PCR reactions. Successful amplification of the 147-bp *ual* fragment was observed in reactions containing up to 5 µL of urine, whereas amplification was abolished when 6 µL was included (Figure 4B). Similar results were obtained for *K. pneumoniae* and *P. mirabilis* (data not shown), indicating that urine up to 20% of the reaction volume did not cause detectable PCR inhibition.

Direct PCR amplification from unprocessed urine

To assess the feasibility of direct PCR, urine samples artificially inoculated with approximately 400-500 *P. mirabilis* cells/µL were used as PCR templates without

prior DNA extraction. A distinct 225-bp amplification product was obtained, whereas no amplification was detected in the negative urine control (Figure 4C). Comparable amplification profiles were observed for *E. coli* and *K. pneumoniae* (data not shown), demonstrating successful amplification directly from untreated urine.

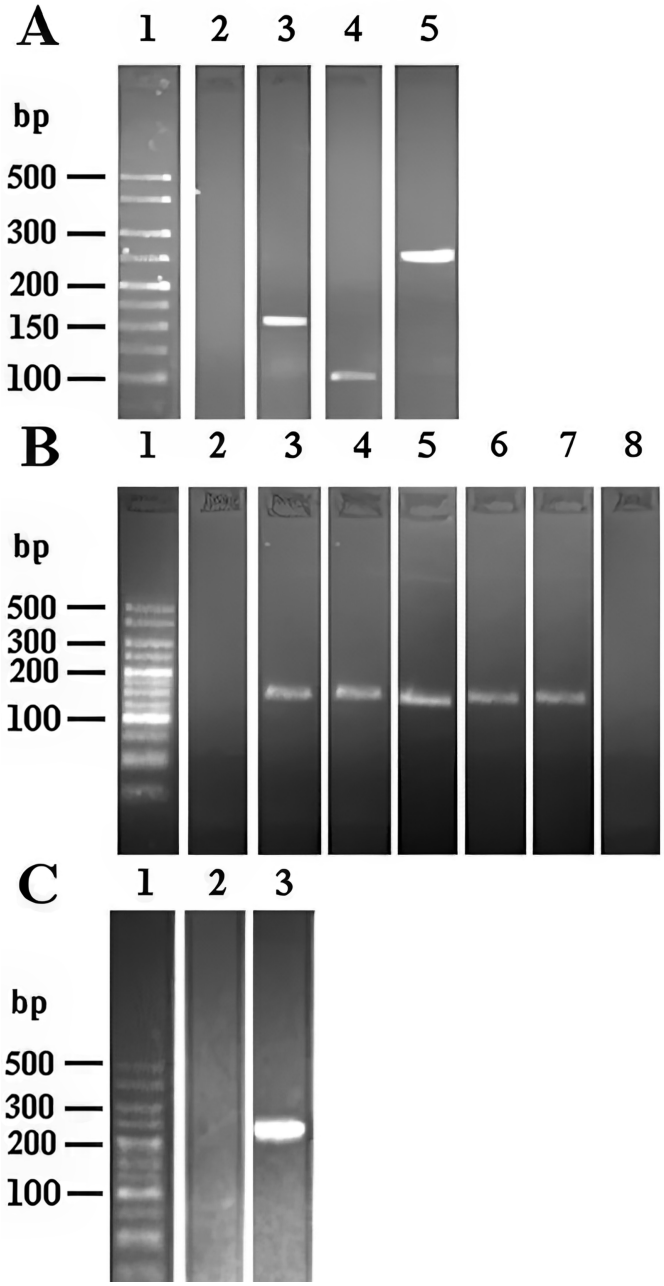

Figure 4: Representative agarose gels of PCR reactions. In panel A, the template used in PCR was DNA purified from urine samples that were positive for *E. coli* (lane 3), *K. pneumoniae* (lane 4), or *P. mirabilis* (lane 5). In panel B, the template used in PCR was different urine volumes inoculated with *E. coli* DNA. One to six µL of urine was tested, and the corresponding reactions were loaded in lanes 3 to 8, respectively. In panel C, the PCR template was 5 µL of *Proteus-contaminated* urine (lane 3). In all panels, lane 1 was loaded with Hyper ladder 100 bp (Bioline) as a size marker, and lane 2 was loaded with a DNA-free PCR control.

Multiplex PCR standardization

Multiplex PCR conditions were optimized to simultaneously detect the three target uropathogens. Both duplex (*E. coli*/*P. mirabilis*) and triplex (*E. coli*/*K. pneumoniae*/*P. mirabilis*) assays produced the expected amplification products (147, 120, and 225 bp, respectively), with no evidence of nonspecific amplification or primer cross-reactivity (Figure 5). Multiplex PCR results were in complete agreement with conventional urine culture for bacterial identification.

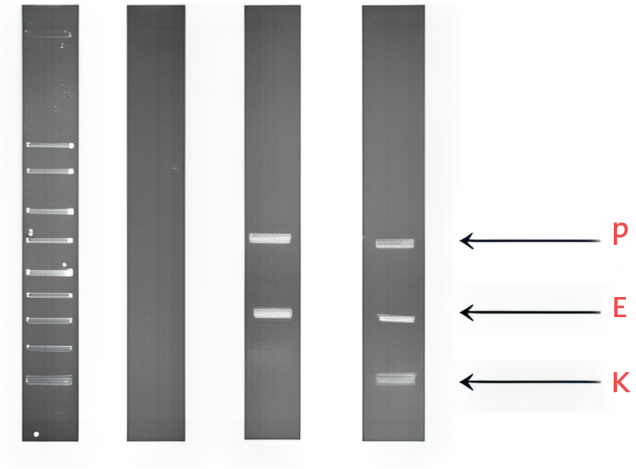

Figure 5: Representative gel of double and triple multiplex PCR of DNA extracted in the presence of urine. The first lane shows the 100 bp marker; the second lane negative control; the third lane double PCR with amplified products of *P. mirabilis* (P) at 225 bp and *E. coli* (E) at 147 bp; the fourth lane, triple PCR with amplified products of *P. mirabilis* (P) at 225 bp, *E. coli* (E) at 147 bp, and *K. pneumoniae* (K) at 120 bp. 1% agarose gel stained with 1.6 µL RedSafe.

Discussion

Urinary tract infections are among the most common bacterial infections worldwide and a major reason for medical consultations and antibiotic prescriptions. *E. coli* predominates, and *K. pneumoniae* and *P. mirabilis* are among the most frequently isolated uropathogens [13, 14]. Timely diagnosis is essential to initiate appropriate treatment, reduce complications, and promote the rational use of antimicrobials. Although urine culture remains the gold standard, it has significant limitations, including turnaround times of 48 hours and reduced sensitivity in patients with prior antibiotic exposure. These limitations can delay critical clinical decisions and encourage the indiscriminate use of broad-spectrum antimicrobials [14, 15], thereby contributing to antimicrobial resistance. In this context, molecular diagnostic techniques have emerged as a promising alternative for the early detection of major uropathogens, with the potential to shorten diagnostic time, reduce reliance on sample processing, and optimize the clinical management of UTIs [16, 17].

In response to these challenges, the present study evaluated a rapid, simple, and low-cost multiplex PCR strategy for the simultaneous detection of the three leading uropathogens, *E. coli*, *K. pneumoniae*, and *P. mirabilis*, that can be applied directly to unprocessed urine and that reproduced conventional culture results with full concordance. Conventional culture, although still the reference standard, typically requires 18–48 h and loses sensitivity after antibiotic exposure [8]. By collapsing identification of the three species into a single reaction and eliminating the DNA-extraction step, the assay shortens the diagnostic workflow to under two hours, with direct implications for empirical prescribing, isolation decisions, and antimicrobial stewardship in the border-hospital setting examined here.

Analytically, the assay behaved as a robust identification tool. Amplification of the species-specific *ual*, *ntrA*, and *ureR* targets was fully concordant with culture across all 56 culture-positive samples, and BLAST verification of each target against the NCBI nucleotide database supports the specificity required to avoid cross-reactivity in polymicrobial urine. The serial-dilution experiment detected as little as ~0.04 pg of genomic DNA, a sensitivity that substantially exceeds the 10^5 CFU/mL threshold of quantitative culture. This analytical margin raises the possibility of detecting sub-threshold bacteriuria in precisely those patients for whom culture is least informative: individuals with recent antibiotic exposure, immunosuppression, or infections caused by slow-growing or fastidious organisms [18]. The full agreement observed here aligns with earlier head-to-head comparisons showing that multiplex PCR is non-inferior to culture for uropathogen detection and recovers organisms that culture misses [19]; our data extend that observation to a low-cost, gel-based format suitable for laboratories without real-time platforms. The duplex and triplex formats resolved the 120, 147, and 225 bp amplicons without overlap or primer cross-reactivity, confirming that the three targets can be co-amplified reliably in a single tube and detected on a standard agarose gel.

The most transferable methodological finding is that urine can serve directly as PCR template, without prior extraction, as long as it does not exceed 20% of the reaction volume (5 µL in a 25-µL reaction). The 94 °C initial denaturation alone lysed bacterial cells at 400–500 cells/µL and released amplifiable DNA, reproducing the thermal-lysis principle previously validated for *Shigella* in food matrices [10]; the 20% ceiling coincides with the urea-mediated polymerase inhibition described for other urinary templates [20], indicating a physicochemical rather than target-specific limit. Eliminating extraction removes reagents (phenol, chloroform, commercial kits), equipment (centrifuges, spectrophotometers), hands-on time, and associated biosafety steps [8], lowering both the economic and infrastructural barriers to molecular diagnostics in public and resource-limited laboratories such as those serving the Reynosa region.

The clinical profile of the cohort reinforces the panel's design. *E. coli* predominated (≈86%), followed by *K. pneumoniae* and *P. mirabilis*, a hierarchy that matches the established epidemiology of community- and healthcare-associated UTIs [1, 13]. The female predominance among positive cases (≈70%) is consistent with the well-described anatomical basis of ascending infection [1, 13]. The *ureR* marker selected for *P. mirabilis* is, moreover, not merely taxonomic: *ureR* encodes the transcriptional activator that controls expression of the urease gene cluster [21], and urease-driven alkalinization of urine underlies the struvite-stone formation and catheter encrustation that define the clinical severity of *P. mirabilis* infection [22]. Detection of *ureR* therefore identifies strains carrying the genetic capacity for this pathogen's most damaging phenotype, adding clinical information beyond species identification.

Because the three target species are recognized zoonotic opportunists, the assay also serves as a surveillance tool within a One Health framework. Cross-species and foodborne transmission has been documented for *E. coli* [5], *K. pneumoniae* [23], and multidrug-resistant *P. mirabilis* [24]. The Reynosa–McAllen corridor, with its binational food supply chains, intensive livestock activity, and continuous cross-border movement, provides conditions under which resistant uropathogenic clones can circulate across human, animal, and environmental compartments [7]. A culture-free reaction that can be deployed close to the point of care provides a realistic means of generating the integrated, comparable data that such surveillance demands. In a binational corridor where multidrug-resistant strains are an emerging concern, shortening time-to-identification also supports more disciplined empirical prescribing, which in turn helps contain the resistance pressure that drives the very transmission dynamics under surveillance.

Beyond their clinical role, the three target species are paradigmatic colonizing opportunistic pathogens whose ecological range spans humans, companion animals, livestock, wildlife, food, and water [4]. This shared ecology has direct molecular consequences: extended-spectrum β-lactamase–producing *E. coli* and *K. pneumoniae* co-occur across human and animal compartments worldwide, and a systematic synthesis of the available evidence confirms that overlapping resistance-gene pools are mobilized between hosts and environments [25]. Direct links between food-producing animals, retail meat, and human uropathogenic *E. coli* have been demonstrated [5], and One Health surveillance has traced multidrug-resistant *K. pneumoniae* [19] and *P. mirabilis* [24] across livestock and human settings. The Reynosa–McAllen corridor

concentrates the conditions that amplify such exchange: binational food supply chains, intensive livestock activity, heterogeneous antimicrobial stewardship across two health systems, and continuous human movement [6, 7], making it a sentinel site for transboundary AMR. Positioned within this framework, the assay reported here is more than a clinical test: because its three targets are precisely the shared zoonotic opportunists, and because it amplifies directly from minimally processed samples, it is readily transferable to the non-clinical matrices that One Health surveillance requires: veterinary specimens, retail-food rinses, wastewater, and agricultural drainage. A single, low-cost, standardized reaction deployable across all these compartments offers a realistic mechanism to generate the comparable, cross-sectoral data on which integrated, binational stewardship depends.

A prospective One Health design built on this baseline would apply the same reaction in parallel to clinical urine, companion-animal and livestock samples, retail meat, hospital and municipal wastewater, and irrigation/agricultural drainage of the Río Bravo–Rio Grande basin, pairing detection with AMR-gene targets and selective genomic typing to quantify uropathogen and resistance flow across the human–animal–environment interface of the border corridor.

Conclusions

Urinary tract infection in the Reynosa–McAllen border region is caused predominantly by *E. coli, K. pneumoniae,* and *P. mirabilis*. These three zoonotic opportunistic pathogens make UTIs a natural target for One Health surveillance. Here, a multiplex PCR assay amplifying directly from unprocessed urine matched conventional culture while markedly shortening turnaround time, establishing it as a rapid, sensitive complement to culture rather than a replacement. These results offer a methodological baseline that future work can extend beyond the clinic to livestock, food chains, and wastewater, integrating genomic analyses to map transmission and guide binational public health action.

Declarations and supplementary information

Acknowledgments: EVP was supported by AUIP, while CMSS and ASR were supported by SECIHTI grants no. 627529 and 676211, respectively.

Author contributions: Conceptualization, JGEF and EMGO; methodology, CMSS, EVP and EMGO; validation, EVP and CMSS; formal analysis, JGEF, EMGO, and EVP; investigation, CMSS; writing, original draft preparation, CMSS, ASR and EVP; writing, review and editing, ASR and CMSS. All authors have read and agreed to the published version of the manuscript.

Funding source: This study was supported by Secretaria de Investigacion y Posgrado (SIP) through project number 20261096.

Conflict of interest statement: The authors declare no conflicts of interest.

Ethics statement: The study protocol was approved by the Institutional Review Board of the Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional (Approval No. CBE/006/2020). All procedures complied with the Declaration of Helsinki and the Mexican Official Standard NOM-007-SSA3-2011 governing clinical laboratory practice.

Data availability: All data used and analyzed in this study are available upon request from the corresponding author.

Supplementary materials: Not applicable.

Declaration regarding the use of generative AI and AI-assisted technologies in the writing process: OpenAI GPT was used to assist language editing. All scientific content, interpretation, and final manuscript revisions were performed and verified by the authors, who assume full responsibility for the manuscript.

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