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POINT-OF-CARE AND LABORATORY-BASED MOLECULAR DIAGNOSTICS FOR MULTIDRUG- RESISTANT UROPATHOGENS: STRATEGIES, CHALLENGES, AND OPPORTUNITIES

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ABSTRACT

Multidrug-resistant (MDR) uropathogenic *Escherichia coli* (UPEC) remains the leading cause of complicated urinary tract infections (UTIs) and represents a significant threat to effective clinical management. Conventional culture-based diagnostics are limited by prolonged turnaround times, often resulting in delayed targeted therapy and inappropriate empiric antibiotic use. Rapid molecular in vitro diagnostic (IVD) platforms have emerged as a promising solution, enabling early pathogen identification and detection of key antimicrobial resistance (AMR) determinants directly from urine specimens.

This review summarizes current evidence on the clinical, technological, and economic impact of rapid molecular diagnostics for MDR UPEC. Multiple studies demonstrate strong concordance between genotypic resistance markers and phenotypic susceptibility for major mechanisms such as extended-spectrum β-lactamases and carbapenemases, with time to appropriate therapy reduced from days to hours. These gains support improved patient outcomes, enhanced antimicrobial stewardship, and reduced healthcare utilization. However, important limitations persist. Molecular assays cannot fully capture the expanding diversity

of resistance mechanisms, and genotype–phenotype discordance remains a challenge for resistance mediated by regulatory mutations, efflux systems, porin alterations, or low-level gene expression. Consequently, molecular diagnostics should complement rather than replace phenotypic susceptibility testing.

Implementation barriers, particularly in low- and middle-income countries, include high costs, infrastructure requirements, supply chain limitations, and workforce constraints—concerns that are especially critical given the disproportionate MDR UPEC burden in these settings. Future directions include expanded resistome panels, decentralized and point-of-care molecular platforms, phenotype–genotype integration using advanced analytics, and linkage with digital AMR surveillance systems.

In conclusion, rapid molecular diagnostics represent a pivotal advancement in MDR UPEC detection and UTI management. Their strategic integration into clinical workflows and surveillance frameworks will be essential to maximize clinical benefit and support global antimicrobial resistance containment efforts.

KEYWORDS: Uropathogenic *Escherichia coli*; multidrug resistance; rapid molecular diagnostics; urinary tract infections; antimicrobial resistance; in vitro diagnostics; antimicrobial stewardship; point-of-care testing.

INTRODUCTION

1.1 Global Burden and Epidemiology of Urinary Tract Infections (UTIs)

Urinary tract infections (UTIs) represent one of the most common infectious diseases globally, accounting for over 150 million cases annually and contributing substantially to morbidity, antibiotic consumption, and healthcare expenditure worldwide.[1,2] *Escherichia coli*, particularly uropathogenic E. coli (UPEC), is responsible for nearly 70–90% of community-acquired and a significant proportion of healthcare-associated UTIs.[3] The predominance of UPEC arises from its distinct virulence factors—including adhesins (e.g., P fimbriae, type 1 fimbriae), siderophore systems, toxins, and immune evasion mechanisms—that enable efficient colonization, persistence, biofilm formation, and recurrent infections within the urinary tract.[4,5]

A growing global concern is the rapid emergence of multidrug-resistant (MDR) UPEC strains, particularly extended-spectrum β-lactamase (ESBL) and carbapenemase producers.

Surveillance reports across Asia, Africa, Europe, and Latin America consistently demonstrate ESBL- producing UPEC prevalence rates exceeding 30–60% in many regions, with LMICs showing the highest burden due to unregulated antibiotic access and limited stewardship infrastructure.[6–8] Carbapenem-resistant Enterobacteriaceae (CRE), though less common, are rising steadily and linked to significant mortality, especially among high-risk groups such as renal transplant recipients, diabetic patients, and individuals with indwelling catheters.[9,10] These antimicrobial resistance (AMR) trends correlate strongly with increased treatment failures, escalation to more toxic or costlier therapies, higher recurrence rates, and complications including pyelonephritis, urosepsis, and bloodstream infections [11]. Additionally, MDR UTIs contribute to prolonged hospital stays and impose substantial economic burdens on healthcare systems globally.[12]

Conventional diagnostic workflows—comprising urine culture, organism identification, and phenotypic antimicrobial susceptibility testing (AST)—require 48–72 hours to produce definitive results. During this interval, clinicians often initiate empiric broad-spectrum antibiotics, which may be inappropriate and inadvertently accelerate AMR selection pressure.[13,14] This delay contributes to therapeutic mismatches, increased resistance propagation, and suboptimal patient outcomes. Therefore, the integration of rapid diagnostics capable of simultaneously detecting UPEC and key AMR determinants (such as ESBL genes, carbapenemases, and fluoroquinolone resistance markers) is urgently needed. Early, targeted therapy informed by rapid molecular detection can significantly reduce inappropriate antibiotic use, optimize stewardship efforts, shorten time to clinical improvement, and curb the spread of resistant strains.[15–17] The subsequent sections of this review outline a comprehensive framework for the conceptualization, design, analytical validation, clinical evaluation, and real-world deployment of an advanced IVD platform tailored for UTI management.

2. Biology of UPEC and Mechanisms of Antimicrobial Resistance

2.1 UPEC Virulence and Adaptation to the Urinary Tract

Uropathogenic *Escherichia coli* (UPEC) constitute a specialized pathotype within extraintestinal pathogenic *E. coli* (ExPEC), possessing a unique complement of virulence determinants that facilitate urinary tract colonization, persistence, and immune evasion.[18,19] Successful UPEC infection involves coordinated deployment of adhesins, toxins, iron-acquisition systems, metabolic adaptations, and biofilm formation. Fimbrial adhesins—

particularly type 1 fimbriae (FimH adhesin) and P fimbriae (PapG)—mediate adhesion to urothelial receptors, driving colonization, invasion of bladder epithelial cells, and formation of intracellular bacterial communities (IBCs) that protect the bacteria from host immunity and antimicrobial exposure.[20,21] Type 1 fimbriae enable binding to mannosylated receptors on superficial bladder epithelial cells, while P fimbriae mediate ascending infection toward the kidneys, contributing to pyelonephritis. Toxins such as α-hemolysin (HlyA) and cytotoxic necrotizing factor-1 (CNF1) induce host cell lysis, promote inflammation, and facilitate bacterial dissemination within the urinary tract.[22] These toxins modulate host cytoskeletal pathways, impair neutrophil function, and enhance bacterial survival.

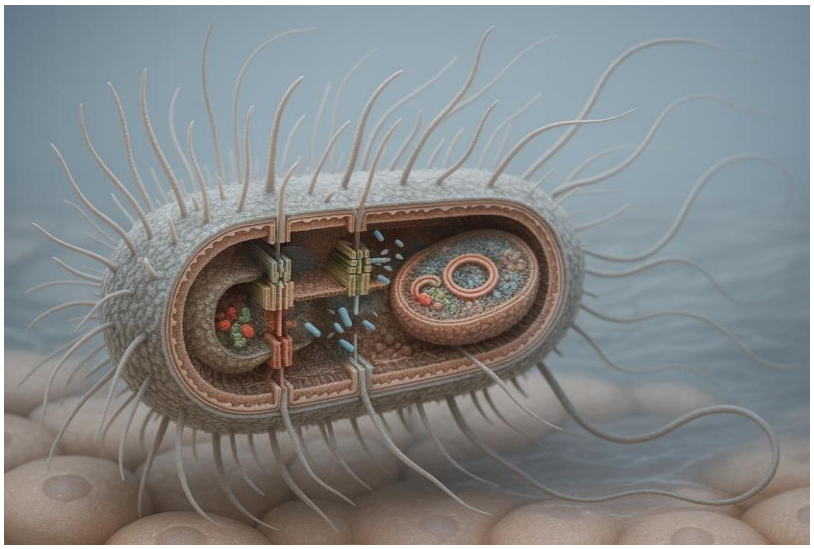

Figure 1: High-resolution 3D rendering of UPEC demonstrating bacterial architecture and antimicrobial resistance mechanisms, including efflux pumps, modified porins, beta-lactamase activity, and plasmid-borne resistance genes, in the context of urinary tract colonization

Iron acquisition is critical in the iron-limited urinary environment. UPEC expresses multiple siderophore systems, including enterobactin, salmochelin, yersiniabactin, and aerobactin, allowing efficient iron scavenging and conferring competitive advantage over commensal strains.[23] UPEC also exhibits a strong ability to form biofilms, facilitated by curli fibers, cellulose, and extracellular matrix components. Biofilms enhance persistence on uroepithelial surfaces and indwelling devices, contributing to chronicity and recurrence of UTIs.[24] Together, these virulence traits promote adhesion, invasion, intracellular survival, evasion of innate immune responses, and transition from cystitis to pyelonephritis, establishing UPEC as the dominant global cause of UTIs.

2.2 Emergence and Diversity of Antimicrobial Resistance in UPEC

UPEC has demonstrated rapid and diverse acquisition of antimicrobial resistance (AMR), driven by selective antibiotic pressure and extensive horizontal gene transfer.[25] Resistance mechanisms include enzymatic degradation, altered drug targets, efflux pump overexpression, and impermeability due to porin loss. β-lactam resistance is predominantly mediated by plasmid- encoded extended-spectrum β-lactamases (ESBLs) such as CTX-M, TEM, and SHV, which hydrolyze oxyimino-cephalosporins. CTX-M-15 and CTX-M-14 represent the most globally widespread variants.[26] Increasing reports of carbapenemase-producing UPEC, including NDM, KPC, OXA-48, and VIM, further complicate treatment, especially in regions with high antibiotic misuse.[27] Fluoroquinolone resistance arises primarily from mutations in the quinolone resistance-determining regions (QRDRs) of gyrA and parC, often accompanied by plasmid mediated quinolone resistance genes such as qnr, aac(6′)-Ib-cr, and qepA.[28] These mechanisms frequently co-occur with ESBL genes, leading to multidrug-resistant (MDR) phenotypes.

Aminoglycoside resistance results from aminoglycoside-modifying enzymes (AMEs)—such as acetyltransferases, nucleotidyltransferases, and phosphotransferases—and from 16S rRNA methyltransferases that confer high-level resistance to nearly all aminoglycosides.[29]

The mobilization of these determinants is facilitated by mobile genetic elements, including conjugative plasmids, integrons, transposons, and insertion sequences, allowing rapid horizontal gene transfer both within UPEC strains and to other Enterobacteriaceae.[30] This genetic fluidity underpins the global expansion of high-risk lineages, most notably E. coli ST131, ST69, ST405, and ST1193, which are associated with ESBL production, fluoroquinolone resistance, and enhanced virulence traits[31,32] The combination of virulence and multidrug resistance in these internationally disseminated clones exacerbates the clinical burden of UTIs, contributing to treatment failure, recurrence, and limited therapeutic options.

3 Diagnostic Targets and Molecular Detection Principles

Rapid detection of uropathogens and their antimicrobial resistance determinants is essential for guiding effective therapy, particularly given the rising prevalence of multidrug-resistant (MDR) urinary tract infections (UTIs). Molecular diagnostics provide a timely and sensitive alternative to culture-based methods, provided they target clinically relevant genetic markers. Among β- lactamase producers, extended-spectrum β-lactamases (ESBLs) remain central. Genes such as bla_{CTX-M}, bla_{TEM}, and bla_{SHV} are

widely used as molecular targets due to their high prevalence in *Escherichia coli* and other Enterobacterales associated with UTIs.[33,34] For carbapenem resistance, key genes include bla_{NDM}, bla_{OXA-48-like}, bla_{KPC}, and bla_{VIM}, which are increasingly reported in both hospital- and community-acquired UTI isolates.[35] Non–β-lactam resistance markers include fluoroquinolone resistance, primarily linked to mutations in gyrA and parC, and aminoglycoside resistance, often due to aac(6')-Ib-cr, aadA, or 16S rRNA methyltransferases such as armA.[36] These targets are increasingly incorporated into multiplex panels due to their therapeutic relevance.

A variety of molecular platforms support the detection of these markers. Conventional PCR and real-time qPCR offer high sensitivity and specificity, with the capability to multiplex several targets and provide quantitative readouts.[37] Isothermal amplification methods, such as Loop- Mediated Isothermal Amplification (LAMP), offer rapid (<1 hour) detection with minimal instrumentation, making them suitable for point-of-care (POC) settings.[6] Recent LAMP-based urine assays have demonstrated 95–96% sensitivity and specificity compared to culture.[38]

CRISPR-based detection systems (e.g., Cas12 or Cas13) provide exceptional specificity through guide RNA–directed cleavage and can detect low-copy or single-nucleotide variants, offering potential for rapid, high-specificity detection of carbapenemase or ESBL genes.[39,40] These assays can be adapted to lateral-flow or fluorescence-based formats and integrated with amplification methods such as RPA or LAMP for rapid, portable diagnostics. Multiplexing strategies that combine organism identification (e.g., *E. coli*–specific markers like fimH or uidA) with simultaneous detection of multiple resistance genes significantly enhance diagnostic efficiency and facilitate early, targeted therapy. Such integrated approaches are increasingly critical for timely management of MDR UTIs, where culture-based methods may be too slow or insensitive.

4 IVD Kit Architecture: From Sample to Result

Designing an in vitro diagnostic (IVD) kit for urinary tract infection (UTI) and antimicrobial resistance (AMR) detection requires engineering that accommodates the biological variability of urine while ensuring analytical robustness and clinical usability.

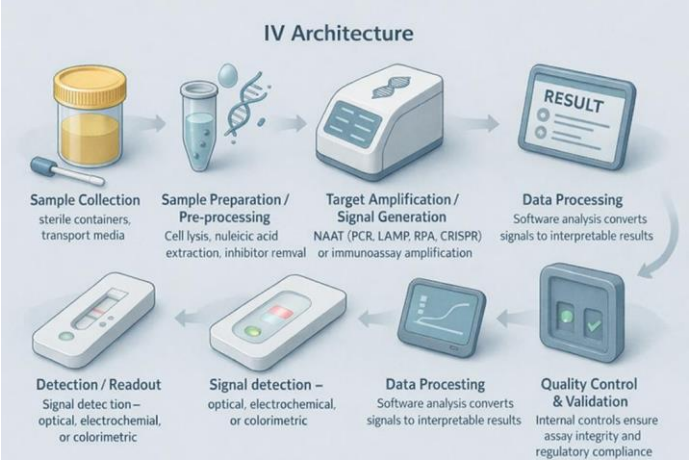

Figure 2: Seven-Stage Diagnostic Flow in Modern IVD Systems.

1. Sample Collection and Pre-Processing

Urine samples exhibit significant variability in volume, bacterial burden, and inhibitory substances such as salts, urea, and endogenous DNases, which can interfere with molecular amplification.[41,42] Low colony-forming units (CFUs) and polymicrobial infections further complicate diagnostics, as conventional culture may miss fastidious or low-abundance pathogens.[43]

To overcome these challenges, sample workflows typically include

- Pre-concentration through centrifugation, filtration, or microfluidic devices, improving direct-from-urine nucleic acid yield.[44]
- Efficient nucleic acid extraction using chemical lysis buffers, bead-based purification, or magnetic bead-based systems optimized for urine matrices.
- Assessment of extraction efficiency, often via internal control amplification, to detect inhibitory substances or extraction failure.[41]

These steps minimize false negatives and stabilize assay performance across patient samples.

2. Reagent System and Internal Quality Controls

A robust IVD kit requires rigorous internal and external quality controls to ensure reliability and reproducibility

- Internal Amplification Control (IAC): Typically a non-competitive heterologous sequence that verifies amplification success and detects inhibitors.[1]
- Positive controls: Containing known bacterial DNA or synthetic targets to confirm reagent integrity.

- Negative controls (NTC): Detect potential contamination in reagents or workflow.
- Extraction controls: Validate DNA recovery and sample-processing efficiency.[45]

These quality systems align with regulatory expectations for molecular diagnostics and support consistent performance in diverse clinical settings.

3. Assay Architecture & Platform Design

3.1 Multiplex Molecular Assays

- Multiplex PCR and qPCR enable simultaneous detection of uropathogens and AMR genes, enhancing diagnostic speed and comprehensiveness compared to culture.[46,47]
- Isothermal methods (e.g., LAMP) offer low-cost, equipment-minimal amplification suitable for decentralized or point-of-care testing.[48]
- Digital PCR provides high sensitivity and absolute quantification, particularly beneficial for low-copy targets.[49]

3.2 Hardware Requirements

- Laboratory deployment: High-throughput thermocyclers and automated nucleic acid extraction instruments are preferred.
- Point-of-care (POC) deployment: Compact closed-cartridge platforms reduce contamination risk and require minimal operator training.[50]

Design considerations include

- Closed-tube amplification to maintain biosafety
- Automated interpretation of amplification curves
- Integration with Laboratory Information Systems (LIS) and Electronic Health Records (EHR) for streamlined clinical workflow.[51]

4. User-Centric Design, Biosafety, and Real-World Usability

- Lyophilized reagents enable ambient storage and stability, critical for low-resource settings.[52]
- Minimal pipetting and closed systems reduce operator error and contamination risk.
- Rapid turnaround times (30–90 minutes) offer substantial improvement over the 24–72 hours required for culture plus antimicrobial susceptibility testing (AST).[43]
- Simplified biosafety requirements are possible because DNA amplification occurs in closed systems.[46]

Cost, portability, and regulatory compliance are essential considerations to enable implementation in diverse clinical environments, from tertiary hospital laboratories to remote outpatient or POC facilities.

5 Optimization Strategy

Analytical optimization of a urine-based molecular diagnostic platform requires systematic refinement at every stage—from primer/probe design to workflow robustness.

Primer and Probe Design: Primers and probes should ensure broad allelic coverage of target resistance determinants, including diverse bla_{CTX-M}, bla_{TEM}, bla_{NDM}, and bla_{OXA-48-like} variants, while maintaining high specificity and minimal off-target binding. Inclusion of multiple alleles is essential due to substantial sequence heterogeneity of AMR genes arising from global dissemination and local evolutionary pressures.[34] Reaction Condition Optimization: Reaction parameters, such as magnesium ion concentration, annealing temperature, polymerase chemistry, and buffer additives, must be experimentally optimized to maximize amplification efficiency and inhibitor tolerance. This is particularly important for urine matrices, which contain salts, urea, and other PCR inhibitors that can reduce sensitivity.[42]

Analytical Sensitivity and Limit of Detection (LOD): LOD studies should be performed using well-characterized bacterial strains spiked into pooled or individual human urine at defined concentrations. These experiments assess sensitivity, linearity, and reproducibility under clinically relevant conditions.[53,54] Multiplexing Considerations: Multiplex molecular assays require rigorous cross-reactivity and interference testing. Balanced amplification efficiency, primer–dimer assessment, and careful spectral separation of fluorescent probes are necessary to prevent competitive inhibition among multiple targets.

Workflow Robustness and Point-of-Care Readiness: Lyophilized reagents improve stability, eliminate cold-chain requirements, and facilitate transport to decentralized settings. Integrated cartridge-based systems that combine extraction, amplification, and detection in a closed format reduce operator variability, contamination risk, and training requirements, enhancing feasibility for point-of-care deployment.

6 Validation Framework and Clinical Performance

A rigorous validation framework is essential to ensure that a rapid molecular IVD assay for

UPEC and antimicrobial resistance (AMR) meets international diagnostic standards and delivers clinically meaningful improvements in patient care. Validation should integrate analytical, clinical, and operational components, aligned with CLSI guidelines (M52, M100), ISO 15189 requirements, and best practices in molecular diagnostic development.

6. 1. Analytical Validation

1.1 Limit of Detection (LOD)

LOD must be quantitatively established for each organism-specific and AMR gene target using serial dilutions of reference strains spiked into pooled human urine. Modern NAAT-based UTI assays typically achieve LOD values of 10^2–10^3 CFU/mL, correlating with clinically significant bacteriuria.[55,56] Determination should follow ≥20 replicates per dilution per CLSI EP17-A2 standards.

1.2 Linearity and Dynamic Range

Linearity is determined using 5–7 logarithmic dilutions of quantified genomic material. Amplification efficiency between 90–110% and R^2 ≥0.98 is required for quantitative assays.[57,58]

1.3 Reproducibility and Precision

Intra-assay, inter-assay, inter-operator, and inter-instrument reproducibility should be evaluated across multiple days and laboratory settings. Coefficient of variation (CV) <5% for Ct or time- to-signal is acceptable.[59]

1.4 Inclusivity and Exclusivity Panels

Inclusivity testing should cover genetically diverse UPEC isolates (e.g., ST131, ST1193, ST405). Exclusivity testing should include non-UPEC *E. coli*, commensal Enterobacterales, and non-uropathogenic bacteria such as *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Enterococcus faecalis*. Performance aligns with EUCAST AMR diagnostic guidance and FDA molecular panel evaluation principles.[60,61]

1.5 Interference and Robustness

Common urinary interferents (blood, nitrites, leukocyte esterase, urea, phenazopyridine) should be evaluated to ensure assay robustness. Lyophilized reagents and cartridge-based workflows enhance stability and reduce operator error.[62]

6. 2. Clinical Validation

2.1 Study design Clinical performance should be assessed using prospective and/or retrospective urine specimens, ideally across multiple centers, benchmarked against culture, phenotypic AST, and genotypic confirmation (PCR, Sanger, WGS). A minimum of 200–500 clinical urine specimens is recommended for robust statistical power.[63] Studies should include both symptomatic and asymptomatic bacteriuria cases and high-risk populations (e.g., transplant recipients, catheterized patients).

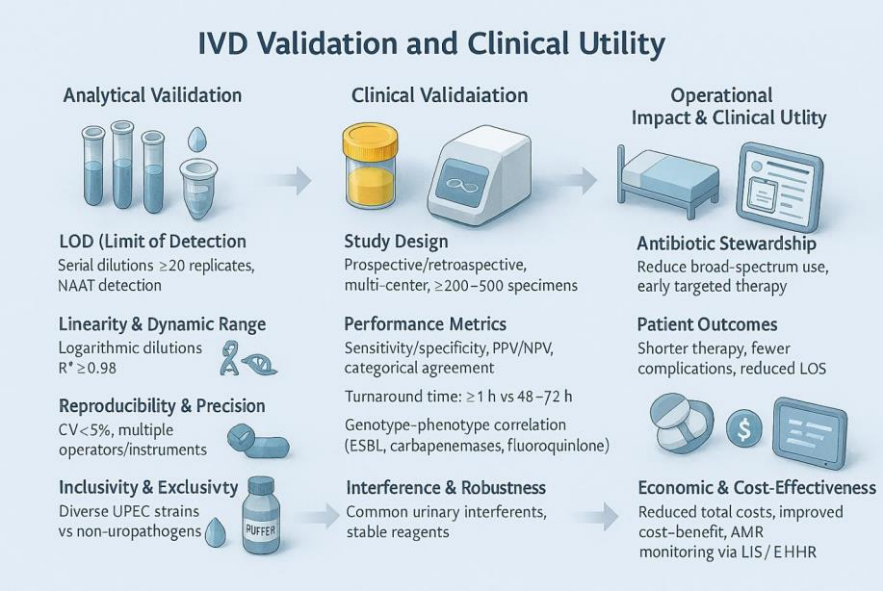

Figure 3: Comprehensive IVD Validation Pathway: Analytical, Clinical, and Operational Dimensions.

2.2 Key Clinical Performance Metrics

- Sensitivity and specificity (target ≥90–95% for organism ID; ≥95% for AMR gene detection)
- Positive and negative predictive values (PPV/NPV)
- Overall categorical agreement with phenotypic AST
- Cohen's κ for genotype–phenotype concordance
- Turnaround time comparison: rapid IVD (≤1 h) vs culture (48–72 h)

Genotype–phenotype correlation should be established for ESBL (e.g., bla_{CTX-M}), carbapenemases (bla_{NDM}, bla_{OXA-48-like}, bla_{KPC}), and fluoroquinolone resistance determinants (gyrA/parC). High concordance (>90–95%) has been reported in recent NAAT-based UTI validation studies.[64,65]

3. Operational Impact and Clinical Utility

3.1 Antibiotic Stewardship Metrics

Rapid molecular diagnostics reduce unnecessary broad-spectrum antibiotic use, especially fluoroquinolones and carbapenems.[66,67] Expected benefits include reduced empiric exposure, earlier pathogen-directed therapy, and decreased antibiotic days of therapy (DOT).

3.2 Patient Outcome Improvements

Clinical utility studies demonstrate shortened time to effective therapy, lower rates of treatment failure and relapse, reduced incidence of pyelonephritis and urosepsis, and shorter hospital length of stay (LOS).[68]

3.3 Economic and Cost-Effectiveness Impact

Health economic analyses show net cost savings from reduced LOS, lower total antibiotic expenditures, and favorable cost–benefit ratios when rapid diagnostics prevent complications.[69,70] Integration with LIS/EHR supports automated reporting, real-time AMR monitoring, and hospital-level surveillance aligned with WHO GLASS recommendations.[71]

7 Quality Systems and Regulatory Compliance

The development of an in vitro diagnostic (IVD) assay for rapid detection of UPEC and antimicrobial resistance determinants must be anchored within internationally recognized quality management, risk-control, and regulatory frameworks. A comprehensive quality system ensures assay reliability, reproducibility, patient safety, and global market acceptability.

ISO 13485 provides the foundational Quality Management System (QMS) requirements for medical device manufacturers, mandating structured processes for design control, documentation, verification/validation, supplier qualification, manufacturing consistency, and corrective/preventive actions (CAPA).[72] For molecular diagnostics, adherence to ISO 13485 not only ensures robust quality oversight but also facilitates regulatory submissions across multiple jurisdictions.[73] ISO 14971 outlines principles for risk management throughout the device lifecycle, requiring hazard identification, risk estimation/evaluation, mitigation strategies, and continuous monitoring.[74] Molecular IVDs—especially those guiding antimicrobial therapy—must undergo rigorous risk analysis encompassing false-negative/false-positive outputs, reagent instability, contamination, operator error, and software interpretation pathways. Mitigation elements include redundant internal controls,

locked assay parameters, and fail-safe reporting mechanisms.[75] Human-factors engineering and usability design are governed by IEC 62366, which mandates systematic evaluation of user–device interaction to minimize use-related errors.[76] For point-of-care (POC) or decentralized testing, streamlined workflow steps, intuitive interfaces, and integrated error prompts reduce operator burden and enhance assay reliability in real-world clinical settings.[77]

Regulatory Oversight varies by region

European Union (EU): IVDs must comply with the In Vitro Diagnostic Regulation (IVDR, EU 2017/746), requiring clinical evidence, post-market performance follow-up (PMPF), and expanded manufacturer responsibilities.[78]

- United States (FDA): Oversight includes pathways such as 510(k), De Novo, or PMA depending on device risk class, requiring analytical and clinical performance data consistent with FDA guidance.[79]
- India (CDSCO): IVDs are regulated under the Medical Device Rules (MDR) 2017, with voluntary alignment to ICMED 13485 for quality certification.[80]
- WHO Prequalification (WHO-PQ): Ensures suitability for global health procurement, especially for LMIC deployment, with specific requirements for performance and design of molecular assays.[81]

Additional regulatory requirements include design verification/validation, biocompatibility, software validation, stability/shelf-life studies, transport/shipping simulation, and lot-to-lot consistency evaluations.[82] Stability testing (accelerated and real-time) must follow ICH Q1A(R2) guidelines to ensure reagent robustness across temperature and humidity extremes.[83]

Post-market surveillance (PMS) ensures continued safety and performance. Under IVDR and FDA requirements, PMS incorporates trend analysis, complaint handling, vigilance reporting, and periodic safety updates.[84] Molecular diagnostics for antimicrobial resistance must include change control mechanisms to update resistance gene panels in response to evolving epidemiology (e.g., emerging carbapenemase variants or plasmid-mediated resistance) while maintaining validated assay integrity.[85]

8 Implementation, Economics, and Health Impact

The deployment of rapid molecular IVD platforms for UTI diagnosis spans diverse clinical

environments, including centralized hospital laboratories, emergency departments, outpatient clinics, and decentralized or point-of-care (POC) facilities—particularly in resource-limited regions where delays in conventional diagnostics disproportionately affect outcomes. Effective implementation requires flexible workflow integration, compatibility with existing laboratory infrastructure, and user-friendly interfaces that minimize operator variability. Seamless connectivity with Laboratory Information Systems (LIS) or Electronic Health Records (EHR) ensures rapid reporting and supports antimicrobial stewardship interventions by enabling real- time clinical decision-making.[86–88]

Economic evaluations consistently demonstrate that although molecular assays incur higher per- test costs compared with traditional culture-based diagnostics, they offer substantial downstream benefits. Early identification of UPEC and associated AMR determinants reduces inappropriate empiric therapy, shortens time to targeted treatment, and minimizes progression to severe complications such as pyelonephritis or sepsis. These improvements translate into reduced hospital length of stay, lower rates of readmission, and substantial savings in overall healthcare expenditure.[89–93] Cost-effectiveness models indicate that rapid molecular testing becomes economically favorable when the prevalence of multidrug resistance exceeds 10–15%, a threshold already surpassed in many regions globally.

Furthermore, implementation at the point of care has demonstrated significant clinical impact. Rapid diagnostics facilitate immediate therapy optimization, reducing reliance on broad- spectrum agents such as fluoroquinolones and carbapenems, thereby enhancing antimicrobial stewardship outcomes.[94] In low-resource settings, simplified cartridge-based or lyophilized assays reduce the need for cold-chain storage and highly trained personnel, enabling decentralized testing with high diagnostic yield.

Ultimately, the integration of rapid UTI IVD systems improves individual patient outcomes and contributes to broader public health goals by reducing resistance selection pressure, improving treatment appropriateness, and optimizing resource utilization across the health system.

9 Discussion, Limitations, and Future Directions

Rapid molecular in vitro diagnostic (IVD) platforms for multidrug-resistant (MDR) Uropathogenic *Escherichia coli* (UPEC) represent a major advancement in the timely diagnosis and management of urinary tract infections (UTIs). By enabling accelerated

organism identification and detection of key resistance determinants, such assays have the potential to significantly improve patient outcomes, guide antimicrobial stewardship, and reduce healthcare- associated costs. Multiple studies demonstrate that early genotypic AMR detection correlates strongly with phenotypic resistance patterns for major mechanisms such as ESBLs and carbapenemases, shortening time-to-targeted therapy from days to hours.

Despite these advantages, several important limitations exist. No molecular assay can fully capture the evolving diversity of AMR mechanisms. Emerging resistance genes—such as mcr variants for colistin resistance or novel β-lactamases—may fall outside fixed diagnostic panels, necessitating continuous updates.[95,96] Genotype–phenotype discordance remains a recognized challenge, particularly for resistance mediated by regulatory mutations, efflux pumps, porin alterations, or low-level gene expression that may not be detected by nucleic acid amplification tests (NAATs).[97] Such discrepancies can lead to misclassification of susceptibility, especially for fluoroquinolone and aminoglycoside resistance.

While molecular IVDs reduce turnaround times, deployment in low- and middle-income settings remains constrained by cost, infrastructure requirements, supply chain limitations, and workforce training. These barriers are critical given the disproportionately high MDR UPEC burden in LMICs.[98]

Future directions include

- Expanded resistome panels incorporating mcr, ermB, blaOXA-181, and emerging carbapenemases.[99]
- Portable or fully instrument-free point-of-care devices using LAMP, RPA, or CRISPR-based systems for decentralized testing.[100]
- Phenotype–genotype bridging approaches, using transcriptomics or machine learning to improve susceptibility prediction.[101]
- Integration with digital AMR surveillance platforms (GLASS, regional public health networks) to support real-time monitoring and outbreak detection.[102]

In summary, rapid molecular assays for UPEC represent a transformative innovation with substantial clinical and public health relevance. While limitations related to resistance coverage, molecular–phenotypic discrepancies, and resource barriers persist, ongoing technological advances and global investment in diagnostic infrastructure are likely to enhance their performance, affordability, and overall impact.

10 CONCLUSION

Rapid molecular in vitro diagnostics have transformed the detection of multidrug-resistant uropathogenic Escherichia coli by enabling early organism identification and resistance profiling, substantially reducing time to targeted therapy. For major resistance mechanisms such as ESBLs and carbapenemases, strong genotype–phenotype concordance supports their clinical value in improving patient outcomes and antimicrobial stewardship.

However, these assays are not comprehensive. The evolving UPEC resistome, persistent genotype–phenotype discordance, and limited detection of resistance mediated by regulatory or structural mechanisms necessitate cautious interpretation and continued reliance on phenotypic susceptibility testing. In parallel, barriers to implementation in low- and middle-income settings—where MDR UPEC burden is greatest—remain a critical challenge.

Future impact will depend on expanded resistance coverage, decentralized point-of-care platforms, and integrative approaches combining molecular diagnostics with phenotypic prediction and surveillance systems. Strategic deployment of these technologies will be central to precision UTI management and global antimicrobial resistance containment.

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