

Optimizing Detection of Antimicrobial-resistant *Escherichia coli*: A review of traditional, Rapid and emerging Diagnostic Technologies

Mawahb Hatem Mones¹, Sarab Murad Kadhem² and Ibrahim Faisal Ibrahim³

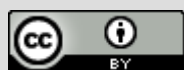
^{1,2}Departments of Basic Science, College of Dentistry, Al-Iraqia University, Baghdad, Iraq,
³Al-Naji University, College Of Health & Medical Technologies.

*Corresponding Author: Mawahb Hatem Mones

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ABSTRACT: Antimicrobial resistance (AMR) in *Escherichia coli* (*E. coli*) represents a major threat to global healthcare because it increases the occurrence of severe urinary tract infections and bloodstream infections and gastrointestinal infections which lead to higher rates of sickness and death. The conventional method for antimicrobial susceptibility testing (AST) provides dependable results; however, its testing process requires 18 to 24 hours of incubation time which creates a delay in making vital medical choices. The review presents a systematic assessment which compares traditional methods to rapid phenotypic testing and molecular testing and newly developed diagnostic methods. The automated phenotypic systems which include VITEK 2 and MALDI-TOF mass spectrometry systems demonstrate faster result delivery because they achieve testing results within 4 to 8 hours and less than 4 hours respectively and they maintain testing accuracy above 92 percent. The real-time PCR molecular assay achieves its highest level of specificity which exceeds 99 percent within a testing period of 1 to 4 hours. The rapid diagnostic tests face challenges from their expensive upfront costs and their complicated operating procedures; however, these tests need to become part of clinical workflows because they provide essential support for precision antibiotic treatment which helps prevent broad-spectrum antibiotic misuse while strengthening worldwide antimicrobial stewardship programs.



Keywords: *Escherichia coli*, Rapid phenotyping, PCR, diagnostic methods, MDR

1. INTRODUCTION

The antimicrobial resistance (AMR) is regarded as one of the most critical issues in the global arena of health concerns, as it decreases the efficacy of widely-used antibiotics and contributes to the increased morbidity and mortality rates across the globe. One of the most prevalent kinds of bacterial pathogens that are frequently isolated and cause the most varied kinds of diseases is *Escherichia coli* that can cause urinary tract infections, bloodstream infections and gastrointestinal diseases. The rise and spread of multidrug resistant strains experienced in recent years has made it more difficult to treat in most parts of the world and made treatment more difficult [1,2]. *E. coli* can acquire the various forms of resistance, including the production of extended-spectrum β -lactamases (ESBLs) and carbapenemases, which constitute a significant health care issue in healthcare systems. These resistance mechanisms are often plasmid-carried, and are interchangeable between different bacteria, such that resistance genes can quickly spread through bacterial populations[3]. The standard method for detecting resistance still depends on conventional antimicrobial susceptibility testing methods. However, these methods usually require a relatively long time due to incubation periods, which may delay appropriate treatment decisions. Therefore, there is an increasing need for faster diagnostic methods that can accurately detect antimicrobial resistance in clinical isolates of *E. coli* [4].

In recent years, several new diagnostic techniques have been developed to improve the speed and accuracy of resistance detection. These involve rapid methods of susceptibility testing, molecular, and mass spectrometry-based techniques. Such methods have shown promising results in identifying resistant strains more quickly compared to conventional laboratory methods [5].

The aim of this review is to outline recent developments in rapid diagnostic methods of identifying antimicrobial resistance in *E. coli*, with a special focus on conventional methods, new technologies, and their clinical consequences.

2. MECHANISMS OF ANTIMICROBIAL RESISTANCE IN *ESCHERICHIA COLI*

2.1 ENZYMATIC DEGRADATION OF ANTIBIOTICS

One of the most important mechanisms of (AMR) in *Escherichia coli* is the production of enzymes that inactivate antibiotics before they reach their targets. Out of these enzymes, the most significant role in the resistance to β -lactam antibiotics is attributed to β -lactamases. Extended-spectrum β -lactamases (ESBLs) are capable of hydrolyzing a wide range of β -lactam antibiotics, including third-generation cephalosporins. Moreover, KPC, NDM, and OXA-type carbapenemases have become prime agents of carbapenem resistance in *E. coli*, with dire consequences on treatment options in clinical care [6].

The spread of carbapenem-resistant *E. coli* (CRE) in the Middle East has become alarming, mainly due to the NDM and OXA-48-like enzymes. The recent surveillance findings in Iraq and the neighboring countries have shown that the number of multidrug-resistant isolates in the clinical facilities has risen sharply, and so the localized rapid screening procedures are necessary to stem the nosocomial spread [7]. The recent reports on the world have evidently pointed at the rise of plasmid-mediated colistin resistance, especially the *mcr-1* gene in *E. coli* isolates. The clinical risk assessment demonstrates that colistin usage serves as the final treatment option for most patients who have carbapenem-resistant infections. Scientists currently focus on global surveillance efforts to enhance their ability to identify mobile genetic elements.[8].

2.2 TARGET SITE MODIFICATION

E. coli bacteria exhibit resistance through their ability to alter the sites which antibiotics target. The major bacterial targets which include DNA gyrase and topoisomerase IV show decreased fluoroquinolone binding through gene mutations which affect their encoding genes. The same way as ribosomal proteins and ribosomal RNA undergo modifications, bacteria develop resistance against multiple antibiotic classes which include aminoglycosides and macrolides. Bacteria acquire the ability to survive in the presence of antimicrobial agents which would normally disrupt their cellular functions through these genetic alterations. [9].

2.3 EFFLUX PUMP SYSTEMS

Efflux pumps function as membrane transport proteins which operate to force antibiotics out of bacterial cells. *E. coli* contains resistance-nodulation-division (RND) family efflux systems which include the AcrAB-TolC efflux pump that plays a vital role in multidrug resistance. Their overexpression may lead to reduced susceptibility to various antibiotic groups including tetracycline, fluoroquinolones and chloramphenicol [10].

2.4 REDUCED MEMBRANE PERMEABILITY

The other significant resistance mechanism is reduction in permeability of bacterial outer membrane. Modifications at the outer membrane porins, especially of OmpF and OmpC, can greatly diminish the uptake of antibiotics in *E. coli*. The alteration or loss of these porin channels restricts access of hydrophilic antibiotics like β -lactam and fluoroquinolones, also adding to the emergence of resistance. This mechanism usually works synergistically with β -lactamase production or efflux pump activity in order to promote multidrug resistance (figure 1) [11].

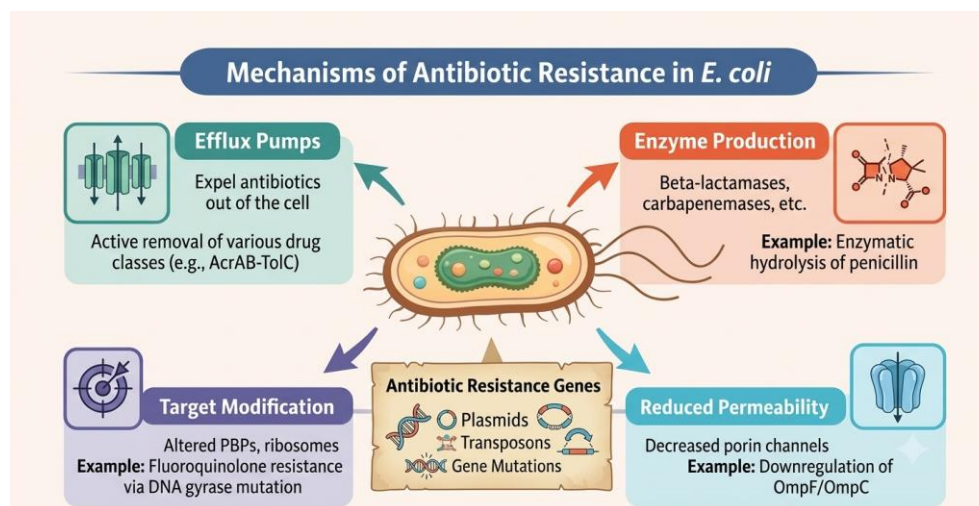


FIGURE 1. - Mechanisms of Antimicrobial Resistance in *E. coli*

3. CONVENTIONAL METHODS FOR DETECTING ANTIMICROBIAL RESISTANCE

Traditional antimicrobial susceptibility testing (AST) remains the most common method of determining resistance in *Escherichia coli* isolates. They are widely used because they are highly reliable, reproducible, and comply with standardized procedures that have been put forth by internationally recognized organizations, such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12,13].

3.1 DISK DIFFUSION METHOD

The Kirby-Bauer method or disk diffusion method is a technique that places agar plate inoculated with the test organism with antibiotic impregnated disk. Measurement and interpretation of the zones of inhibition are carried out after incubation based on the breakpoints. Although this technique is simple and inexpensive, it usually takes 18-24 hours to incubate, which can slow the process of clinical decision-making [14,15].

3.2 BROTH MICRODILUTION

The gold standard of determining minimum inhibitory concentrations (MICs) of antibiotics is deemed to be broth microdilution. This quantitative technique gives accurate data on the vulnerability of bacterial isolates and it enables low-level resistance to be identified. The process requires extensive work and specific tools which make it impossible to implement in every laboratory. [16,17].

3.3 AGAR DILUTION

Agar dilution is an experiment that includes the introduction of different amounts of antibiotics in agar plates, and then, bacterial strains are added to these plates. The method is technically challenging and time-consuming, although accurate and reproducible, which is why it is not a routine method in clinical settings [16].

Though traditional methods offer essential data used to inform therapy, the lengthy processing time and cumbersome process of such assays has prompted the creation of quicker phenotypic and molecular diagnostic methods to identify antimicrobial resistance in *E. coli* in a more efficient manner [18].

4. RAPID PHENOTYPIC METHODS

To overcome the shortcomings of the traditional antimicrobial susceptibility testing, especially the long turnaround time, rapid phenotypic techniques have been established. Such techniques offer quicker outcomes with a high reliability and reproducibility, thus gaining more significance in clinical microbiology [17,18].

4.1 AUTOMATED SUSCEPTIBILITY TESTING SYSTEMS

Optical or biochemical detection determined microbial growth in the presence of antibiotics is automated systems like VITEK 2 (bioMérieux) and BD Phoenix. Such platforms are capable of creating susceptibility profiles within 4-8 hours, which is much quicker than the traditional approaches. They have been extensively used in clinical laboratories with routine testing of *E. coli* and other *Enterobacteriaceae* [19,20].

4.2 CHROMOGENIC MEDIA

Using chromogenic media, it is possible to quickly identify resistant bacterial colonies due to changes in color as a result of a particular enzymatic activity. As an example, chromogenic ESBL or carbapenemase media allow presumptive identification of resistant *E. coli* isolates in 18-24 hours, which can offer earlier information on infection control and treatment [21,22].

4.3 MICROFLUIDIC-BASED ASSAYS

Microfluidic technologies reduce the size and automate AST, allowing high-throughput phenotypic testing using only small amounts of reagents. By measuring the bacterial growth in microchannels under controlled conditions, these systems can reduce the time of detection to as little as 2–4 hours. Recent research has shown that they are potentially accurate and high throughput in detecting resistance in *E. coli* clinical isolates [23].

In general, there is a balance created by rapid phenotypic methods, which are accurate and fast and able to provide timely information to inform antimicrobial therapy, yet they do not compromise the reliability of traditional testing methods.

5. MOLECULAR DIAGNOSTIC APPROACHES

Detection of antimicrobial resistance in *Escherichia coli* has been revolutionized by molecular diagnostic methods which have offered quick and very specific determination of resistance genes. These methods, as opposed to phenotypic, directly identify genetic determinants making their turnaround time shorter and allowing them to be used in early clinical decision-making [24,25].

5.1 POLYMERASE CHAIN REACTION (PCR)

Using PCR, it is possible to amplify and identify certain resistance genes, including those that encode extended-spectrum β -lactamases (ESBLs), carbapenemases, and other resistance determinants. Traditional PCR may take only a few hours to get results and is commonly applied to clinical laboratories to detect selected resistance [26,27].

5.2 REAL-TIME PCR (QPCR)

The amplification and detection steps are combined in real-time PCR, thus gene copies can be quantified. The method enhances sensitivity, specificity and minimizes time spent on-hand. qPCR assays have also been effectively used to identify ESBL and carbapenemase genes in clinical *E. coli* isolates rapidly [28,29].

5.3 WHOLE-GENOME SEQUENCING (WGS)

WGS gives detailed information about the resistome of bacterial isolates, determining known and novel resistance genes. Whereas WGS used to take several days, the current development of sequencing technology and bioinformatics pipelines can now enable resistance profiling to be done within 24-48 hours. The technique is priceless in investigating outbreaks, surveillance, and informing treatment in multidrug-resistant *E. coli* infections [30].

5.4 DNA MICROARRAY

With DNA microarrays, it is possible to identify many resistance genes at once. Using a hybridization of bacterial DNA to gene-specific probes, this technique enables screening of resistance determinants in high-throughput in one assay. Microarrays find application in research and epidemiological studies, but have yet to be adopted clinically owing to cost and complexity [31]. Molecular techniques are an addition to the quick phenotypic techniques, which offer effective identification of resistance mechanisms and aid in prompt decisions regarding antimicrobial stewardship in clinical practice.

6. RAPID DETECTION USING EMERGING TECHNOLOGIES

The latest technological innovations have resulted in new ways of rapidly detecting antimicrobial resistance in *Escherichia coli*. These new technologies are designed to circumvent the shortcomings of traditional and current high-speed phenotypic/molecular technologies, and can provide quicker outcomes, increased sensitivity, and possibly point-of-care application [32,33].

6.1 BIOSENSORS

In biosensor based assays, biological recognition components, e.g. enzymes, antibodies or nucleic acids, are used to provide specific markers of resistance. Electrochemical and optical biosensors have also been designed which can

detect β -lactamase and carbapenemase-producing *E. coli* quickly, typically in 1-3hours. Such systems can achieve high sensitivity, specificity and can be miniaturized and automated[34].

6.2 NANOTECHNOLOGY-BASED DETECTION

Gold nanoparticles, quantum dots and magnetic nanoparticles are among nanomaterials that have been incorporated in antimicrobial resistance detection platforms. Such Nano-systems are used to improve signal detection, decrease assay time, and enable multiplexing of detection of a number of resistance genes at a time. The recent research has shown that they can identify ESBL and carbapenemase genes in *E. coli* in a few hours and with high accuracy [35,36].

6.3 MALDI-TOF MASS SPECTROMETRY

A new and influential method of rapid phenotypic identification of resistance has become matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. MALDI-TOF can identify resistant strains of *E. coli* by the analysis of certain protein or enzyme patterns in less than 4 hours, as an example, the hydrolysis of carbapenems. This technology is being used in clinics with increasing frequency in the clinical laboratory to conduct rapid AST and surveillance [37,38]. The developing technologies which operate in their early stage and their restricted laboratory testing show high potential to improve infection resistance detection and patient management and antimicrobial stewardship programs.[39].

7. DIAGNOSTIC TECHNOLOGIES, PERFORMANCE METRICS.

The table presents essential performance metrics which include sensitivity, specificity, and turnaround time (TAT) for both traditional and modern diagnostic technologies. [40].

Table 1. - The Comparison of the Performance Metrics of the Conventional and Rapid Diagnostic Methods

Method	Sensitivity (%)	Specificity (%)	Turnaround Time (TAT)
Disk Diffusion	94-99%	95-98%	18-24 Hours
VITEK 2 / BD Phoenix	92-98%	91-96%	4-8 Hours
Real-Time PCR \ qPCR	>98%	>99%	1-4 Hours
MALDI-TOF MS	95-100%	94-98%	1-4 Hours
Biosensors (WGS)	90-97%	92-96%	1-3 Hours

8. CLINICAL IMPLICATIONS OF RAPID DETECTION

The fast detection of antimicrobial resistance in *Escherichia coli* creates important effects for healthcare. The timely detection of resistant strains(such as mcr-1 or ESBL genes) enables clinicians to customize antimicrobial therapy in real time and minimizes the use of broad-spectrum antibiotics and enhances patient outcomes [41,42]. The antimicrobial resistance in *E. coli* should be considered through the prism of One Health, since the resistant strains often cross the border between humans, livestock, and water bodies. The clinical field requires rapid diagnostic devices as essential tools which also serve environmental monitoring purposes to prevent agricultural runoff from contaminating the entire food supply with resistance genes. [43,44].

8.1 GUIDING APPROPRIATE THERAPY

Early identification of resistance allows focused antimicrobial treatment, reducing the risk of treatment failure and avoiding the emergence of other resistant strains. It has been revealed that quick AST can minimize unsuitable empirical treatment, minimize hospitalization, and reduce deaths in patients with bloodstream infections due to resistant *E. coli* [45].

8.2 INFECTION CONTROL AND PREVENTION

Early detection of multidrug resistant *E. coli* helps to take measures of infection control like isolation or cohorting to avoid nosocomial transmission. The timely identification is especially critical in the environment of intensive care units, where the risk of outbreaks is quite high [45].

8.3 THE EFFECT ON ANTIMICROBIAL STEWARDSHIP.

Quick testing approaches can facilitate antimicrobial stewardship initiatives, offering expedient data on resistance, directing de-escalation of broad-spectrum antibiotics, and decreasing unnecessary exposure to antibiotics. The strategy is useful in preventing the development and transmission of resistance in healthcare facilities [46,47].

8.4 ECONOMIC AND PUBLIC HEALTH BENEFITS.

Even though some of the innovative rapid detection techniques are more expensive in the short term, they are also linked with less expenditure in healthcare as fewer complications, fewer hospitalization, and better management of patients. In a larger sense, timely detection of resistance plays a role in surveillance of the health of people and smart policy-making [48].

In general, implementing rapid diagnostic methods into the daily clinical experience is important in enhancing the outcomes of therapy, managing the transmission of resistant *E. coli*, and facilitating the worldwide response to antimicrobial resistance.

9. CHALLENGES AND LIMITATIONS

Although the rapid detection technologies have made a tremendous progress, there are a number of challenges and limitations that inhibit its application in clinical practice. These challenges influence the adoption, precision and cost-efficiency of quick diagnostic approaches to *Escherichia coli* [49,50]. An essential problem in rapid diagnostics is the hetero resistance, in which sub-populations of *E. coli* have various levels of susceptibility. Although molecular techniques indicate the existence of a gene, they do not necessarily indicate the actual phenotype expression and it may result in failure of treatment [51].

9.1 COST AND RESOURCE REQUIREMENTS.

Numerous new rapid diagnostic systems such as molecular assays, biosensors and nanotechnology-based systems are demanding special equipment and trained personnel. The training extends until October 2023 because you have received instruction on this period. The system becomes unworkable for low-resource healthcare settings because its initial expense together with its operational expenses reach excessive levels. The traditional phenotypic testing methods which use disk diffusion testing maintain cost efficiency at approximately two to five dollars per test but they need prolonged time periods for their execution. The direct testing expenses of rapid molecular assays which include qPCR testing and automated platforms exceed 25 dollars and reach 100 dollars or more. The clinical laboratory managers need to analyze the immediate costs of diagnostic instruments against the total savings which their tests will bring to hospital operations because faster test results will decrease testing expenses and hospital resources will be used more efficiently. [52].

9.2 TECHNICAL AND ANALYTICAL LIMITATIONS

Rapid diagnostic tests face limitations because their performance depends on two factors- their sensitivity measurement and their ability to identify new resistance genes. The phenotypic rapid techniques face limitations because they cannot identify rare resistance mechanisms, while molecular techniques can only detect specific genetic markers. [52,53].

9.3 STANDARDIZATION AND REGULATORY APPROVAL

Fast detection techniques need standardized procedures together with regulatory acceptance to achieve their required reliability and reproducibility. The different methods and interpretative criteria together with the reporting standards used by laboratories lead to clinical testing results that vary between facilities. [54].

9.4 MEDICAL WORKFLOW IMPLEMENTATION

The introduction of rapid diagnostics into the daily laboratory processes and clinical decision-making may be complicated. The clinicians require successful communication of quick outcomes which must be integrated with electronic health records and antimicrobial stewardship programs to achieve maximum clinical impact. [54]. The clinical advantages of rapid detection technologies require both problem resolution and effective implementation management to achieve optimal results for patient care and antimicrobial stewardship..

10. FUTURE PERSPECTIVES

Antimicrobial resistance detection in *Escherichia coli* currently progresses at high detection rates which will change through advances in technological and genomic and computational methods. Future developments need to provide better diagnostic tests which will work faster and more accurately while remaining affordable and accessible to all

users. Existing limitations of diagnostic tests will be solved through future technological developments according to figure2. [55, 56].

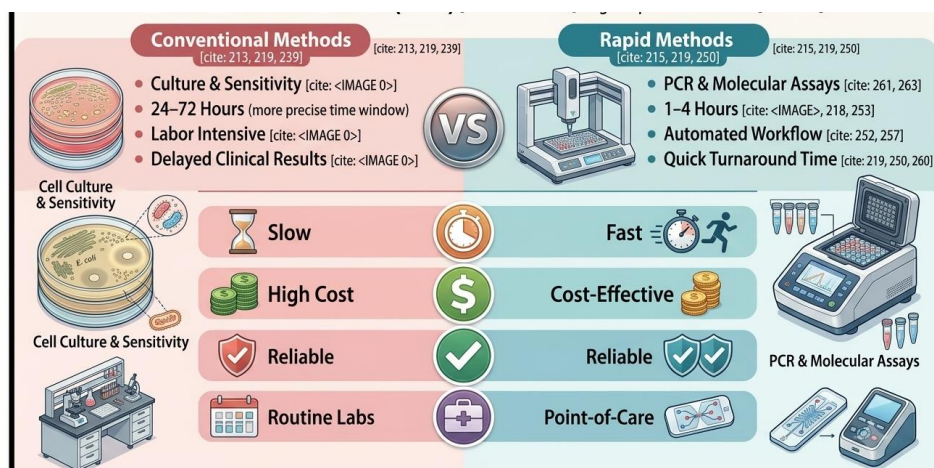


FIGURE 2. - Comparison between Conventional Detection Methods and Rapid Detection Methods

10.1 POINT-OF-CARE TESTING

The diagnostic tests which function at patient locations and use actual patient specimens to determine resistance patterns show great potential for success. The POC tests provide results within a few hours which enables doctors to make instant treatment choices during outpatient care and in facilities with limited medical resources.[57].

10.2 INTEGRATION OF MULTI-OMICS APPROACHES

Researchers can use the combined study of genomics and transcriptomics and proteomics and metabolomics to study resistance mechanisms and find methods to detect resistance. Multi-omics can determine and detect new resistance genes and forecast new patterns of resistance before they can spread.. [58].

10.3 ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING

AI and machine learning algorithms could join more intricate datasets of both molecular and phenotypic measures, although this is not always possible to include in regulatory submissions. Such tools might enhance the predictive accuracy, optimize assays and enable real-time analysis of resistance profiles [59]. Although AI and machine learning have the potential to transform the field of predicting MIC values and resistance phenotypes, the main limitation is the high computational effort, and specialized bioinformatics skills are required to analyze the multifaceted genomic data. The diagnostic frameworks of the future should incorporate the user friendly software interfaces to enable these sophisticated tools to be accessible to the ordinary clinical microbiologists [60,61].

10.4 ECONOMIES OF SCALE AND ACCESSIBILITY

The next area of research needs to be to reduce the cost of rapid diagnostic platforms to allow it to be accessed by a significant number of people in low and middle-income countries. The world needs small, cheap and convenient devices to finish the full surveillance and control of the antimicrobial resistance [62].

The future of rapid diagnostic testing for *E. coli* will achieve three goals through improved testing speed and accuracy and better test availability. These advancements will lead to better patient treatment and more effective antibiotic use and improved health results for people worldwide.

11. CONCLUSION

The rapid detection of antimicrobial resistance in *Escherichia coli* helps doctors treat patients and control infections while helping the world tackle the increasing spread of resistant bacteria. The development of the phenotypic, molecular and new diagnostic technologies has also contributed a lot as far as the reduction of a turnaround time, accuracy and clinical decision making is concerned.

Although the traditional ones are still valid, fast techniques, such as automated systems, chromogenic media, molecular assays, biosensors, nanotechnology-based solutions, and MALDI-TOF mass spectrometry have significant advantages in speed and accuracy. The issues like cost, technical complexity, standardization, and integration in clinical workflows have to be tackled in a manner that will be most effective. Some of the future developments that are

projected to happen include, point-of-care diagnostics, merging of multi-omics and universal accessibility which will eventually be able to identify resistance in a timely and affordable and precise manner. These technologies need to be even more implemented and optimized to lead to the correct therapy, foster antimicrobial stewardship practices, and enhance patient outcomes on the international level.

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CONFLICTS OF INTEREST

This is to confirm that the review article titled Traditional and modern methods, their clinical applications, & associated challenges for Antimicrobial-Resistant *Escherichia coli*. Is my original work. The work has never been published before and it is not submitted to be published elsewhere. Any information source and already published materials have been recognized appropriately. I personally responsible to the content, analysis and conclusion that are provided in this review.”

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