

Real-Time Pathogen Detection Technologies and Their Impact on Antibiotic Prescribing in Emergency Departments: A Review Study, Advances, Challenges, Limitations and Innovations

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Abstracts

Pathogen detection technology has transformed ED practices by identifying infectious agents quickly and accurately enough to eliminate issues such as delayed diagnosis, inappropriate antibiotic prescriptions, and AMR. There are molecular diagnostics, of which polymerase chain reaction (PCR), CRISPR-Cas systems, next-generation sequencing (NGS), and point-of-care devices are a few examples; they assure high sensitivity as well as specificity, making antimicrobial stewardship, de-escalation of broad-spectrum antibiotics, and targeted AM therapy feasible. Further new emerging trends are AI-driven diagnostics, biosensors, and microfluidic platforms. The further smoothing of the detection process comes in the way of automating workflows, combining heterogeneous datasets, and achieving high-speed decision-making processes. The specificity of these innovations lies with the pathogen; these have the multiplexing, short turnaround times to assure early diagnosis and treatment. It also reduces the usage of empirical treatments, limits the application of antibiotics where they do not need to be

applied, and hence limits progression of AMR. Significant drawbacks include a high implementation cost, integration complexity with an already existing healthcare system, and a gap in terms of training that hampers its general adoption. With such challenges, there are areas of hope, like providing subsidies, research together with focused training, etc. These future directions will involve integrating diagnostics with smartphones, universal biosensors, and AI-based real-time analytics. With innovations promising to democratize diagnostics towards resource-constrained settings, it brings equitability in healthcare delivery. Technologies involving real-time pathogen detection may alter the ED workflow, fight back against the AMR challenge, and bring overall health improvement in a faster manner with rapid, reliable, and actionable diagnostics.

Keywords: Real-time pathogen detection, Emergency department, antimicrobial resistance, Molecular diagnostics, Point-of-care testing, Next-generation sequencing, AI-driven diagnostics, antimicrobial stewardship.

1. Introduction

Rapid and accurate pathogen detection in EDs is very well established in the literature. It is crucial that pathogen identification is combined with susceptibility testing for appropriate antibiotic stewardship and targeted antimicrobial therapy. Identification of the relevant pathogens facilitates the search for an infectious focus and allows de-escalation of empiric antimicrobial therapy. This is very critical especially because of the increased rate of multidrug-resistant pathogens, which require broad-spectrum empiric regimens (Rothe et al., 2019). Increasing the detection rate of bacteremia in EDs can improve patient outcomes. Several research studies have demonstrated that detection of multiple pathogens matches a higher risk of repeated ED visits and more exaggerated antibiotic use compared to instances in which a single pathogen is detected (Mannstadt, 2024). Quick and accurate identification of pathogens can also encourage correct prescribing of antibiotics that could help avoid unnecessary use of the drugs (Rao et al., 2021). New technologies such as point-of-care testing and molecular methods, which include PCR and CRISPR-Cas, have the possibility of being applied for the quick, sensitive, and specific detection of pathogens (Kong, 2023; Xia et al., 2023; Wu et al., 2022). This will overcome the significant limitations that characterize culture-based traditional approaches, which include prolonged turnaround times and high rates of false-positive and false-negative results (Kong, 2023; Zhu et al., 2021). Rapid and accurate detection of pathogens is fundamental for appropriate control of infectious diseases, transmission prevention, and the exclusion of public panic (Kong, 2023; Xia et al., 2023; Song et al., 2021). Early identification and isolation of infected individuals are important to limit human-to-human transmission, and sensitive, high-throughput detection methods are required to achieve this end (Song et al., 2021).

This is one of the major concerns in healthcare regarding the delayed or inappropriate diagnosis of antibiotic prescribing and patient outcomes. Various studies have highlighted all the factors contributing to the issue and its consequences. This includes improper antibiotic prescribing due to incorrect diagnosis. Sometimes, research has demonstrated that doctors misdiagnose patients by prescribing them antibiotics for misdiagnosed viral upper respiratory tract infections as

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bacterial infection. This results in the irrational use of antibiotics that further increases the chances of antibiotic resistance (Alkhamees et al., 2018). Time pressure and inability to make a diagnosis are the other determinants of prescribing antibiotics. In the general dental practitioner's survey done in Australia, the pressures causing uncertainty about the diagnosis increased the prescriptions for antibiotics (Teoh et al., 2019). The opposite was also observed in Wales. A study there reported that the odds of the prescription of antibiotics by a dentist were ten-fold if clinic time was limited. Sometimes, inappropriate or delayed diagnosis may lead to inappropriate prescriptions of antibiotics. According to a study conducted in Jordan, acute RTIs constituted the most common diagnosis associated with antibiotic prescription. Such prescriptions were done with poor adherence to guidelines (Alkhalidi et al., 2021). Studies have shown that at sometimes, doctors prescribe antibiotics to some patients based on misdiagnosis, for example viral upper respiratory tract infection misdiagnosed as a bacterial infection (Alkhamees et al., 2018). This results in the irrational use of antibiotics, and therefore the risk of antibiotic resistance is increased (Alkhamees et al., 2018). A study on delayed diagnosis of ulcerative colitis in South Korea found that two years of delay were associated with increased use of anti-tumor necrosis factor-alpha drugs, which have serious side effects (Kang et al., 2019). A study in the United States has also shown that as the COVID-19 cases started to decline following mass vaccination, antibiotic prescribing for older adults with diagnosed SARS-CoV-2 infection decreased, hinting that better diagnosis sometimes translates to more appropriate usage of antibiotics (MacFadden et al., 2023).

2. Real-Time Pathogen Detection Technologies Overview

Culture is relatively less sensitive and time consuming than the real-time pathogen detection technologies, for instance, real-time PCR. According to Kuret (2024) and Farahani & Taghavi (2016), with real-time PCR, it becomes feasible to detect microorganisms even at low copy numbers; therefore, it results in accurate and rapid diagnosis. These molecular tests have become essential diagnostic tools for bacterial infections due to their improved performance characteristics (Kuret, 2024). However, the traditional diagnostic techniques, like culture, may take more time and labor (Chen et al., 2016). In comparison to the conventional RT-PCR and nested PCR, real-time PCR minimizes the time taken in routine sample analysis (Pojezdal et al., 2017). Moreover, real-time PCR is time and labor-saving compared with traditional PCR (Oh et al., 2016). Specificity and multiplexing are also additional benefits of real-time pathogen detection. Real-time PCR assays have been developed for highly sensitive and specific detection of various pathogens. These include *Xylella fastidiosa*, *Ralstonia solanacearum*, and *Agrobacterium* spp. This technique can also be developed to detect and quantify multiple periodontal pathogens, namely *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, and *Treponema denticola* (Farahani & Taghavi, 2016; Coffey et al., 2016). On the other hand, traditional approaches such as checkerboard hybridization are quite time-consuming and laborious when compared to real-time qPCR (Coffey et al., 2016). Bead-based suspension arrays have also proven to be a promising tool in diagnosis, where one can detect viruses, atypical pathogens, and bacteria in the same reaction, unlike most current detection methods that detect either viruses or bacteria (Chen et al., 2016). Beyond, real-time pathogen-detection technologies

like metagenomics next-generation sequencing (mNGS) are potentially capable to directly identify pathogenic genomes, directly from clinical samples by bypassing culture or some of the traditional methods. That approach would give rapid pathogen identification even for already unknown or uncultivated microorganisms (Morsli et al., 2021).

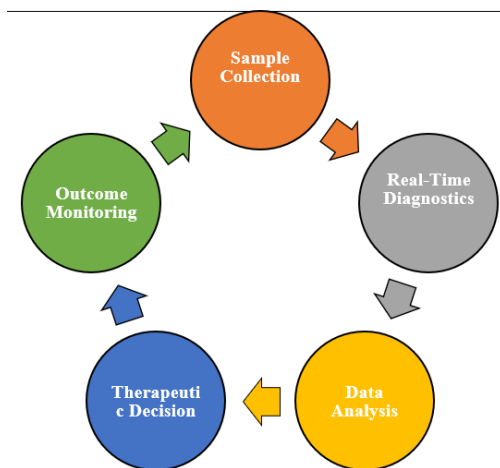


Figure 1. This diagram illustrates pathogen detection process ensures real-time and faster diagnosis and treatment in emergency departments. The starting point of the process includes the collection of samples, during which the patient's specimen is taken with caution to produce reliable results. The samples will be worked on using PCR, CRISPR, or NGS tools among others. These are tools of diagnosis, which can distinguish the pathogen and trace the presence of resistance markers at a very high velocity and precision. The data captured by these tools is later analyzed, often by AI in most cases, to give transparent insights over the infection. Such results enable clinicians to take therapeutic decisions, such as choosing targeted antibiotics instead of broad-spectrum ones, thus providing better use of antibiotics and reducing resistance. The final is outcome monitoring, which tracks the patient's response to treatment and enables readjustment to ensure that the correct adjustments are made. Improved process will enhance patient outcomes, minimize delays, and make ED care a game-changer against antimicrobial resistance.

MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) is a direct, nonchromatographic method that provides fast identification of pathogens. The utility of this technique in rapid characterization of a vast range of microbial species such as clinical pathogens, lactic acid bacteria, nonfermenting bacteria, fungi, plant-parasitic nematodes, and environmental bacteria has been shown (Božik et al., 2021). MALDI-TOF MS has been suggested to be more effective in diagnosing than traditional biochemical techniques (Božik et al., 2021). Another technology that allows one to quickly identify pathogens is the use of high-throughput qPCR. This method ensures that more than one target gene is detected in the same set of reaction conditions; therefore, it is possible to easily detect or identify

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many pathogens in one go (Liu et al., 2019). For example, a TaqMan Array Card developed in the laboratory has been reported to be used for the simultaneous detection of 19 enteropathogens, and there has been a high-throughput assay developed for rapid direct detection of nine pathogens from stools with diarrhea (Liu et al., 2019). This technology has also been used for the concurrent detection and enumeration of various food- and waterborne pathogens such as *Listeria monocytogenes*, *Salmonella Typhimurium*, *Vibrio parahaemolyticus*, and *Clostridium perfringens* (Liu et al., 2019). Apart from that, other molecular tools are LAMP and RPA used in the fast identification of pathogens in diverse uses, among which are agricultural production (Paraguison-Alili et al., 2021). One such kind of technology is said to provide field-friendly alternatives with regard to approaches in and hence ideal candidates toward accomplishing point-of-care molecular diagnostics proposed for ailing patients. Owing to relative simplicity and speed of process plus cost, technology-based on LAMP is very well, in recent times and studies, promising that promise toward revolution for getting that accomplishment toward point-of-care molecular diagnostics (Diego et al., 2019). This work has now focused on improvement of endpoint and, for the first time, near-real-time monitoring of LAMP results to move technology closer to a more realistic point-of-care format in resource-poor endemic areas (Diego et al., 2019). Traditional identification methods, usually from the observation of the microstructures of pathogens, sometimes do not give much surety, but with techniques like LAMP, this contamination can be detected in the field before it causes actual disease in the plants (Siegieda et al., 2021).

Table 1: Real-Time Pathogen Detection Technologies.

Technology	Advantages	Limitations	Examples	References
PCR and qPCR	High sensitivity, specificity, and rapid diagnosis	Requires advanced infrastructure and trained personnel	Real-time PCR, multiplex PCR	Kuret, 2024; Coffey et al., 2016
Next-Generation Sequencing	Comprehensive pathogen and AMR profiling; ability to detect unknown pathogens	High cost, complex data analysis	Illumina sequencing, nanopore sequencing	Morsli et al., 2021; Zhang et al., 2022
Point-of-Care Testing	Quick results; bedside usability	Lower sensitivity compared to lab tests	Immunoassays, lateral flow devices	Maluleke et al., 2021; Shin et al., 2018
CRISPR-Cas Systems	Ultra-sensitive detection: low cost compared to traditional methods	Requires specific expertise and infrastructure	SHERLOCK, DETECTR	Zhang et al., 2021; Song et al., 2021
MALDI-TOF MS	Fast identification across a range of pathogens	Initial investment and training requirements	Biotyper, VITEK MS	Božik et al., 2021; Chen et al., 2016
Biosensor-Based Technologies	High sensitivity and specificity; portable for field use	Variability in detection limits and durability	Electrochemical biosensors, DNA sensors	Wang et al., 2023; Najafabad et al., 2022

This is very pertinent in agriculture, where rapid diagnosis and prevention are essential. High-throughput next-generation sequencing technology was also applied to identify the gene sequences of all eukaryotic microorganisms, which include fungi, from the clinical samples, for instance, of patients suffering with chronic prostatitis, according to Wu et al. (2020). In this approach, a comprehensive screening on pathogens that are challenging in culturing using the old method can be possible. In addition to the technologies listed above, a number of DNA-based techniques that have emerged recently have allowed for rapid and sensitive detection of foodborne pathogens. Generally, molecular techniques, for example, PCR and derivatives, can handle a significant number of samples at one time to provide quick results with high sensitivity (Papatheodorou et al., 2021). Traditional pathogen detection techniques include culture and isolation, with serious limitations such as long detection times and high false positives and false negatives rates (Kong, 2023). To solve these problems, there have been new technologies to come out with some: Polymerase Chain Reaction (PCR), Reverse Transcription (RT)-PCR, among others. These often depend on predicting the candidate target pathogen beforehand and make use of different experimental tools and methods depending on the targets (Kong, 2023).

More recently, CRISPR-based technologies have been developed for rapid and sensitive detection of nucleic acids. These include the Specific High-sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) method that integrates Cas13a with isothermal amplification for the detection of nucleic acids with speed and ultra-high sensitivity (Zhang et al., 2021). Other CRISPR-based platforms have also been developed for rapid and visual detection of nucleic acids as well as for high-throughput and ultra-sensitive diagnostic purposes. Besides the direct detection of pathogens, host-pathogen interaction models, such as the *Dictyostelium discoideum* and zebrafish larvae model, have been used to screen novel anti-virulence molecules against multi-drug-resistant pathogens, including *Klebsiella pneumoniae* (Marcoleta et al., 2018). Such models may clarify the mechanisms of virulence of pathogens and designing new therapeutic strategies.

Another host-pathogen interaction model is the *Caenorhabditis elegans* - *Pseudomonas aeruginosa* infection system. This has been used to create a liquid-based, high-throughput, high-content screening platform to identify novel compounds that limit the ability of the pathogen to kill the host (Anderson et al., 2018). Such an approach will be of value in addressing the threat posed by antimicrobial-resistant pathogens. Molecular diagnostic methods, including PCR and high-throughput sequencing, have also been used in the identification of microorganisms that are difficult to culture, such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Ureaplasma urealyticum* (Sun et al., 2021). Since these pathogens cannot be detected by standard microbiological culture techniques, the only way to detect them is through molecular techniques. The developments over the past years in molecular detection technology have rendered the techniques much more sensitive, specific, and quicker with an automation of the process improved. These developments make for early and rapid detection of pathogens of infectious diseases, like the PCR, isothermal amplification, gene chips, and high-throughput sequencing (Liu et al., 2023). This also changed sericulture. It has the capacity to identify causative agents of diseases of the silkworm quickly and accurately through the molecular techniques developed for the pathogen identification by Deepika in 2024. The effect of this is

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that control measures in the sericulture industry can be effective in use. To increase the screening rate of the target strains, scientists have investigated methods comprising resistant plant varieties and enhancing the endophytic bacteria in the seed samples (Wang et al., 2021).

3. Types of Real-Time Pathogen Detection Technologies

Molecular Diagnostics:

In fact, fast-pathogen detection depends considerably on the use of PCR and some sophisticated variants of it, including qPCR and multiplex PCR. The feature of the method, being very sensitive as well as specific and rapid in identifying infectious agents, has revolutionized the field of clinical diagnostics and microbiology. It's a very powerful molecular method with the ability to exponentially expand particular DNA or RNA strands to detect the smallest particles of pathogenic genetic content (O'Farrell et al., 2019). Therefore, a high sensitivity of PCR in finding the causes as it can trace the prevalence of a disease-causing microorganism even if symptoms have not yet commenced (Kim et al., 2014). This technique has other names such as real-time PCR, an advanced PCR-based approach that provides a relative quantitative estimation of the quantity of genetic material present with the target pathogen (Thopireddy et al., 2021). It comes helpful in infection dynamics so one can estimate how an infection may progress with it for determining the burden or load with respect to the pathogen that may be living with in the host (Li, 2024). qPCR has been applied in the diagnosis of many infectious diseases, including COPD. It has also been perceived to be an extremely sensitive and culture-independent technique for the detection of potential pathogens (O'Farrell et al., 2019). The other advanced form of PCR is multiplex PCR that has enabled many pathogens to be amplified in a single reaction by detecting them (Carpenter, 2024). This is appropriate when the causative agent of a disease is unknown or the number of possible pathogens that need to be tested is broad (Li et al., 2020). Multiplex PCR has been applied in identifying foodborne pathogens using a fast and simultaneous method.

These pathogens include *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Salmonella* spp., and *Staphylococcus aureus* (Kim et al., 2014). Different optimizations have been implemented to increase sensitivity and specificity in PCR-based methods, where BSA is included in a PCR reaction mixture. BSA has been reported to enhance the sensitivity and yield of the PCR reaction in a way that permits the detection of lesser DNA concentrations of the causative material (Kim et al., 2014). Other related applications in plant pathology are the PCR-based approaches employed for identifying pathogens such as *Phytophthora sojae* causing root rot in soybeans. According to Dai et al., (2012), *Phytophthora sojae* was isolated from soybeans. These rapid and sensitive techniques are going to play a critical role in early disease management in plants. Additionally, PCR-based methods have been used in the investigation of SUDI, in which they have been proved to be more sensitive than traditional virus isolation techniques for detecting viral pathogens (Weber et al., 2010). New advanced PCR-based techniques include digital PCR (dPCR) and photothermal-based multiplex nested PCR, which enhance the capabilities of pathogen detection (Li, 2024; Galogahi et al., 2023).

Point-of-Care (POC) Devices:

POCs are crucial because they enhance the detection of pathogens from the bedside by providing a quick and accurate diagnostic tool that can be easily accessed (Maluleke et al., 2021; Moetlhoa et al., 2023). Testing is done at the bedside because it will allow an attending practitioner to decide and begin appropriate treatment in time (Khaleel, 2023). Some of the significant advantages of POC tests include that they provide point-of-care results, which are critical in managing very infectious diseases such as COVID-19 (Maluleke et al., 2021; As such, providing a quick diagnosis, POC tests enable early treatment, hence improving the better management of disease cases and reducing morbidity and mortality rates (Khaleel, 2023). POC tests offer several advantages over traditional laboratory-based diagnostics, which include cost-effectiveness, user-friendly, and deployability outside the healthcare facility (Maluleke et al., 2021; Moetlhoa et al., 2023). This is especially in resource-limited settings where centralized laboratory services may be limited (Moetlhoa et al., 2023).

Different technologies including immunoassays, NAATs, and colorimetric assays may be utilized in the development of POC tests (Shin et al., 2018; Mou et al., 2019). POC tests can identify numerous pathogens including bacteria, viruses, parasites, and fungi and allow clinicians to quickly identify the causative agent and thereby appropriately direct antimicrobial therapy (Bissonnette & Bergeron, 2016; Trick et al., 2021). Multiplex or multiparametric POC tests are very useful since the possibility of achieving clinically relevant genomic information increases because multiple pathogens can be identified simultaneously and their pertinent antimicrobial resistance markers (Bissonnette & Bergeron, 2016). This leads to better and more efficient antimicrobial stewardship and better control over the evolution and spread of resistance genes (Bissonnette & Bergeron, 2016).

POC tests have been effective in diagnosing and controlling a wide range of infections, including HIV/AIDS, TB, and STIs. This would significantly impact patients and surveillance public health in the outcome of a patient as follows: pathogen identification at the point of care as proposed by Trick et al. (2021), Moetlhoa et al. (2023), and Mashamba-Thompson et al. (2016), among others. Even though there are numerous benefits with the application of POC tests, they lack the sensitivity that a test like the gold standard laboratory test, such as bacterial culture, has (Mou et al., 2019).

The current improvement in the sensitivity and specificity of POC tests is also included in the research and development. This extent of improvement will bridge this gap towards giving much more reliable diagnostic capabilities (Mou et al., 2019; Stedtfeld et al., 2012). Besides pathogen detection, POC tests have also been used for other medical applications including monitoring vital parameters, biomarkers measurement, and the detection of genetic markers linked to diseases (Dixon et al., 2016; Stedtfeld et al., 2012). Their convenience and use in various setups of healthcare are further improved through the combination of POC testing with technologies like microfluidics and smartphones (Stedtfeld et al., 2012).

Emerging Next-Generation Sequencing NGS technique with much advanced power can allow very readily real-time full identification of both pathogen and the latter's resistance genes. There have been significant scientific publications and literature in recent years focused on the potential of NGS. Indeed, one main reason that NGS is so robust is that it can test for many agents capable

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of disease causation but generally cannot be isolated or differentiated using more conventional detection technologies (Wang et al., 2020; Zhang et al., 2022). The sensitivity of NGS in the detection of pathogens has been proved to be as high as 80.6% (Zhang et al., 2022), which has turned into a very useful tool for optimizing antibiotic treatment, especially in immunosuppressed patients (Zhang et al., 2022; Wang et al., 2020). In addition, NGS could provide one-step pathogen identification without the long and tedious processes associated with the traditional methods of detection (Wang et al., 2020; Yang et al., 2022). Indeed, this is a significant advantage in the real-time detection of pathogens when NGS can reduce turnaround time compared with conventional methods (Yang et al., 2022).

The wide-ranging capability of NGS in detecting pathogens, including less frequent and challenging microorganisms difficult to culture, has been well proved in clinical conditions, including post-lung transplantations (Lian et al., 2021), lower respiratory infections (Yang et al., 2022), and mixed pulmonary infections (Wang et al., 2019). The following research studies have proven the presence of evidence that NGS outperforms conventional techniques by offering a greater value in diagnosis, especially with infections that are complex or atypical (Lian et al., 2021; Wang et al., 2019). In addition to detecting the causative pathogen, NGS has shown sensitivity in detecting genes that indicate AMR in real-time (Nguinkal, 2024; Gali et al., 2023). This sensitivity will be of good use in the effective surveillance of diseases and outbreak investigation that would lead to more focused control measures (Nguinkal, 2024). NGS has been shown in various settings as part of public health surveillance of AMR within the scope of the laboratories' scope of public health (Gali et al., 2023).

The role of NGS in environmental surveillance, which includes wastewater surveillance, has proven to detect many types of pathogens, including emergent and novel viruses (Smith, 2024). This approach can provide insightful information on community-level patterns of disease transmission and be helpful in pandemic preparedness (Smith, 2024). The uptake of NGS has been low in resource poor regions; however, given the constant advances and drop in cost of NGS technologies, the approach will increasingly be used, and often in combination with more traditional methods, for broad pathogen detection and AMR surveillance (Yang et al., 2022).

Biosensor-Based Technologies:

Biosensors can detect pathogens, especially with high sensitivity and specificity through various mechanisms. An example in this regard is the combining of nucleic acid detection with protein detection on the electrochemical biosensor array as shown by (Mohan et al., 2011). Their work showed the clinical validation of an electrochemical biosensor array for urinary tract infection (UTI) diagnosis that can identify pathogens by detecting bacterial 16S rRNA and evaluate pyuria by detecting lactoferrin (LTF). The assay integrated the possibility of pathogen identification and UTI evaluation on one biosensor array without sacrificing the sensitivity or specificity (Mohan et al., 2011).

Another technique is electrochemical immunosensors that sense urinary lactoferrin, which can be applied as a marker for UTIs. Pan et al. (2010) analyzed the determination of LTF in clinical urine samples with an electrochemical biosensor. A long-established marker for UTIs is pyuria, that is, the presence of white blood cells in urine; LTF, a secretory protein of these cells, has

antimicrobial activity and interferes with pathogens to gain the iron necessary for survival, which is essential (Pan et al., 2010). Nucleic acid-based amperometric biosensors also have promise in terms of disease diagnosis and detection of pathogens due to the high sensitivity and specificity that accompany them. Nagraik et al. (2019) designed an enhanced DNA-based bioassay for the detection of the pathogen *Leptospira interrogans*, which causes the disease leptospirosis. The carboxylated gold electrodes surface was functionalized by immobilizing amino-labeled single-stranded DNA probes through covalent bonding, thus giving evidence for the technique for the detection of pathogens (Nagraik et al., 2019).

The electrochemical biosensors are the devices widely applied to the detection of foodborne pathogens because they offer fast processes, high sensitivity, high specificity, low costs, portability, and point-of-care detection. Recently, Wang et al. reviewed recent advances in electrochemical biosensors for the detection of foodborne pathogens to show their potential application as stand-alone devices in on-site monitoring (Wang et al., 2023). The use of gold nanoparticles in biosensors has also been studied for pathogen detection due to their high specificity, sensitivity, integration, and low detection limits. Najafabad et al. (2022) has also reviewed both colorimetric and non-colorimetric approaches in gold nanoparticle-based biosensors for detection of pathogens (Najafabad et al., 2022). Some studies have focused on the Aptamer-based biosensors detection of pathogenic microorganisms. Wang et al. (2012) have evaluated the application of aptamers in detection of common pathogenic microorganisms such as *Plasmodium*, HIV, *Escherichia coli*, and *Salmonella typhi*. Here, there is interest in developing rapid, sensitive, and inexpensive detection technologies for monitoring and controlling their spread (Wang et al., 2012). The use of the promoter-gene components is also capable in the engineering of biosensors as discussed by (Feng et al., 2018). These genetically engineered promoter-gene biosensors may have wide ranging applications and can be used in various systems, including high throughput transcription factor-acting and anti-tumor drugs screening, over-expression of membrane proteins and enzymes, monitoring nucleocytoplasmic shuttling factors movement and localization, and bacteria and toxic compound density monitoring in food and environment (Feng et al., 2018). Cesewski & Johnson (2020) discussed a critical review of electrochemical biosensors for pathogens detection with their definition and classification according to IUPAC-recommended definitions and classifications. They referred to the different uses of electrochemical biosensors for pathogen detection, such as viruses, bacteria, and eukaryotes, and highlighted the need to pay attention to the target pathogen, sample matrix, biosensor design, fabrication method, measurement format, and biosensor performance (Cesewski & Johnson, 2020). Wu et al. (2019) reviewed the electrochemical DNA biosensors for the detection of foodborne pathogens. Biosensors were categorized based on bioreceptors (antibody, DNA, enzyme, whole-cell, phage) and transducers (electrochemical, piezoelectric, calorimetric, optical). Electrochemical DNA biosensors have the following advantages in detecting pathogens: low detection limit, wide linear dynamic range, and high reproducibility (Wu et al., 2019).

Mokhtarzadeh et al. (2017) did a review on the application of nanomaterial-based biosensors for the detection of pathogenic viruses with comparison of the merits and demerits of different types of detection. They observed that there is a high demand for easy, fast, sensitive, and accurate

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methods of detecting pathogenic agents, and biosensors can be beneficial in these regards (Mokhtarzadeh et al., 2017). Mahari & Gandhi (2022) have surveyed the current advancement electrochemical biosensors have achieved concerning Salmonellosis- the major foodborne disease that occurs. They have mentioned such advantages of biosensors - including an increased sensitivity, specificity, and accuracy, lower in cost, faster response with probable in situ applications; also, portability for detection of Salmonella in foods which makes them an exciting alternative for the determination of Salmonella in food (Mahari & Gandhi, 2022). Yasmin et al. (2016) surveyed the applications of biosensors in food safety and indicated that biosensors can deliver results quickly based on sequential organic reactions, effectively completing the rapid and sensitive detection of foodborne infections, an important goal of biosensor research. The researchers further discussed the utilization of biosensor and nano-scale technologies in food industries for packaging and pathogen detection (Yasmin et al., 2016).

Du et al. 2015 discussed an effective way of catching Salmonella by a 3D biomolecular filter from the liquid flow and its swift identification through automated biosensor measurement system. They underlined the phage's stability in binding capability even at high temperatures and wide pH ranges and its possibility to detect more than one pathogen by using a set of different pathogen-specific phages along with ME biosensors (Du et al., 2015). Sentürk et al. published a review on biosensor-based detection of foodborne pathogens in 2018. Biosensor-based methods have gained a lot of popularity due to their characteristics, including the rapid detection. They highlighted the fact that online biosensor technology can be very beneficial for the food industry in terms of internal process control and the fulfillment of high-quality control standards (Sentürk et al., 2018). An efficient and simultaneous electrochemical approach towards detection of the two major foodborne pathogens, *Escherichia coli* O157:H7 and *Vibrio cholerae* O1, using multifunctional nanoconjugates, was developed by Li et al. (2017). They emphasized that electrochemical biosensors would be very useful in terms of the rapid and sensitive detection of pathogenic bacteria, thus filling the gaps in methods that could detect multiple species of pathogens in a single interface (Li et al., 2017).

AI-Driven Diagnostics:

AI has also mainly contributed towards detecting pathogens in a more efficient and accurate way. All the references enlisted below shall be in support of the statement. AI contributes to the detection of pathogens using the computer vision technique, an image analysis methodology. Numerous research has revealed the capacity of AI-based algorithms to diagnose infectious diseases such as infectious keratitis (Soleimani, 2023), COVID-19 via slit-lamp images (Öztürk et al., 2020; Fontanellaz et al., 2020), and tuberculosis by chest X-rays and CT scans (Acharya et al., 2022). Thereby, AI-based diagnostic tools will provide the rapid and accurate detection of the causative pathogens, with further scope for the timely start of treatment and management of an outbreak (Poblete-Echeverría, 2023; Tran et al., 2021). Besides image analysis, AI and ML can be used to process more comprehensive sources of data for improved pathogen detection. The electronic health records, social media data, among other datasets, may go through the AI algorithms to analyze potential outbreaks and disease patterns (Isiaka, 2024; Panah, 2023). This helps to identify infectious disease threats early and quickly respond to them, which is the core of public health (Parums, 2023; Tran et al., 2021). AI and ML models are known to deal with

huge and complex data sets, thus bringing patterns and trends that are not easy for the naked human eye to see (Tran et al., 2021; Panah, 2023; Tran et al., 2023). Extremely handy in infectious disease surveillance by which AI can scan bulk information emanating from different sources and gives early warnings of the eruption (Isiaka, 2024; Panah, 2023; Parums, 2023). Furthermore, AI-based new drugs discovery and development may rush the development of new medication against infectious diseases and be battle-ready to fight ANDIGEMA (2024). AI can speed up the identification of potential therapeutic candidates in drug discovery while improving the development of successful treatments. Besides that, AI-based public health interventions can be applied to improve the design and implementation of targeted interventions, such as predictive modeling for resource allocation and personalized treatment recommendations (ANDIGEMA, 2024). It will allow for more efficient and precise detection of pathogens because, according to references, AI is a highly effective tool. It would support improvement in surveillance, diagnosis, and management of infectious diseases (Poblete-Echeverría, 2023; Tran et al., 2021; Isiaka, 2024; ANDIGEMA, 2024; Panah, 2023; Parums, 2023).

4. ED Uses

Real-time pathogen-detecting techniques are relevant in providing supportive data in making quicker decisions for diagnosing and then treating a patient in EDs. These real-time PCR and advanced molecular diagnostic technologies have come in with far-reaching benefits to be in comparison with standard approaches on the etiologies of some infectious causes behind the pathophysiology of a host of respiratory illnesses such as an exacerbation of COPD or CAP (Shimizu et al., 2015; Yoshii et al., 2016). One of the key advantages of real-time PCR is that it detects all varieties of pathogens, including viruses, atypical bacteria, and typical respiratory bacterial pathogens in a single test (Shimizu et al., 2015). It has been observed that with this comprehensive approach, the detection rate of infectious etiology of COPD exacerbations was increased from 52% using conventional methods to 88% when real-time PCR was used in combination with the latter (Shimizu et al., 2015). Similarly, in CAP, it has been proven that real-time PCR greatly enhances the sensitivity of pathogen detection in adults who are hospitalized, especially those who have previously received antibiotics (Yoshii et al., 2016). The benefits of real-time PCR do not stop there. Real-time PCR is also useful for the determination of the load of pathogens by creating standard curves and giving useful information to the clinician to make an appropriate treatment decision (Haber et al., 2017). This quantitative nature of real-time PCR is specifically very useful when antibiotics have been given already because it could allow for the detection of the presence of associated bacteria that conventional culture-based techniques would otherwise miss (Yoshii et al., 2017). Along with real-time PCR, some of the other advanced molecular techniques like metagenomic next-generation sequencing (mNGS) have also emerged and have been proven to be successful in rapid pathogen detection (Zeng et al., 2021).

mNGS provides advantages in sensitivity, high throughput and broad coverage that allow it to detect known as well as new pathogens within a given specimen such as cerebrospinal fluid and blood, (Zeng et al., 2021). Early diagnosis supported by mNGS may be contributing to early

Sajidah Taqi Baqer Alshakhs, Radiah Hussain Ali Alsafar, Ali Hussain Alhumud, Ahmed Mohammed Aljubarah, Batool Sadiq Taher Aldahneen, Abdullatif Hussien Khadem AlAbdullatif, Mohammed Ali Ahmed Alkhawaja, Ali Hussain Alhaddad, Zahraa Jafar Alsultan, Iqbal Ibrahim AlAli, Farhan Hameed Albaiji, Aljwharah Abood Nami Aljuaid, Mohammed Awadh S Alharbi, Maha Mubarak Mesfer Aldawsari, Hind Almadi Khalaf Alrowli

intervention of appropriate interventions for treatment. Besides, the newly developed CRISPR/Cas-based biosensing systems have shown excellent performance in nucleic acid detection with high sensitivity, specificity, and simple setups (Li, 2023). These innovative tools have the potential to become important diagnostic instruments in the fight against newly emerging pathogens, such as SARS-CoV-2 (Li, 2023). Besides molecular approaches, other rapid detection methods, such as immunochromatographic test kits, have been used for the detection of specific pathogens, including mastitis-causing streptococci in veterinary medicine (TSUGAMI, 2024). These assays might not be as sensitive as real-time PCR, but they are more straightforward and rapid and more economical to the extent that they can become beneficial in resource-limited areas or for point-of-care testing (TSUGAMI, 2024). Nevertheless, it must be observed that there must be proper validation and benchmarking of real-time pathogen detection tools against traditional methods, even at a pure culture or DNA samples but with clinical specimens for successful deployment into the EDs (Venbrux, 2023). Cost-effectiveness must be weighed before the incorporation of new technologies into mainstream practice (Venbrux, 2023).

One of the major challenges in modern medicine is the differentiation between bacterial and viral infections, since the appropriate treatment and management strategies often depend on the underlying etiology. To address this challenge, researchers have explored various diagnostic tools and biomarkers that can help distinguish between these two types of infections. One of the exciting avenues of research involves markers based on the protein expressed from monocytes known as MxA, myxovirus resistance protein A, which is quickly induced following viral infection, yet persists for an extended time, thereby being indicative of a viral immune response. Studies have shown that MxA is highly sensitive and specific for the differentiation of viral from bacterial infections (Metz et al., 2023; Sambursky & Shapiro, 2015). Among the commonly used biomarkers for differential diagnosis between bacterial and viral infections are WBC count, absolute neutrophil count, ESR, CRP, and PCT. These markers have some useful information, but the specificity is low and increase in bacterial as well as viral infections. These were the reason behind research about diagnostic accuracy that approaches on combining biomarkers may eventually bring improved accuracy (Bozlu et al., 2018).

For example, two markers MxA and CRP have been shown to offer greater sensitivity when used together rather than a single marker especially in distinguishing between viral and bacterial infections (Sambursky & Shapiro, 2015; Self et al., 2017). FebriDx is that test which measures the levels of MxA and CRP, offering a highly diagnostic ability especially for distinguishing between bacterial and viral upper respiratory infection (Self et al., 2017). Apart from the approaches that are biomarker-based, advances in molecular diagnostics have also contributed toward the differentiation between bacterial and viral infections. Rapid on-site methods of viral testing that depend on reverse transcription-polymerase chain reaction, for instance, have been put together to expedite diagnosis, especially concerning the presence of viruses in a sample (Masarweh et al., 2023). Besides, host gene expression-based classifiers have also been promising for differentiating viral from bacterial acute respiratory illnesses (Zaas et al., 2013; Tsalik et al., 2016). Another factor to be considered is co-infection, where both the bacterial and the viral pathogen are present simultaneously. The research studies have shown that respiratory

viral infections predispose people to secondary bacterial infections, hence the need for diagnostic tools that would identify these complex interactions (Choi et al., 2019; Hendricks et al., 2016).

5. Impact on Antibiotic Prescribing Practices

Improved Accuracy:

The following references provide insights into the several different kinds of technologies that might ensure antibiotics are used safely and appropriately: Rapid diagnostic testing-including procalcitonin and respiratory viral panels-has potential to reduce the inappropriate use of antibiotics in aiding towards diagnostic uncertainty about the causes of the respiratory indications. These technologies, unless perhaps bundled with education, AMS direction, and audit, are probably not optimal to achieve these (Timbrook et al., 2017).

Prospective audit and feedback (PAF) approaches can also promote appropriate antibiotic use. A study found that modifying PAF from targeting select IV antibiotics to all IV antibiotics can have a broader impact and avoid shifting resistance from one antibiotic class to another (Langford et al., 2019). Clinical decision support systems will optimize the prescribing process, mainly through auditing of decisions, providing real-time feedback and increasing compliance with antibiotic prescribing guidelines to reduce unnecessary and inappropriate prescribing (Laka et al., 2021). Some studies showed that CDSS can even reduce the duration of antibiotic therapy, length of hospital stay, and the costs (Laka et al., 2021). A new technology, peptide-conjugated phosphorodiamidate morpholino oligomers, enhances the effectiveness of antibiotics by targeting bacterial resistance genes specifically (Ayhan et al., 2016). PPMOs can reduce the doses of antibiotics in combination therapies and potentially target specific genera or multiple genera through sequence-specific targeting (Ayhan et al., 2016). Community pharmacy-based antimicrobial stewardship interventions, such as the TARGET Antibiotic Toolkit, include educating staff, developing patient-facing materials, and completion of checklists by patients and pharmacy staff (Hayes et al., 2022; Parekh et al., 2023). Interventions center on enhancing patient knowledge and involvement and support pharmacists with safety and appropriateness checks. Machine learning and artificial intelligence accelerate diagnosis, including the detection of pathogens and forecasting resistance to antibiotics, thus promoting targeted and appropriate application of antibiotic therapy (Harris, 2023; Yelin et al., 2019). Machine learning and artificial intelligence enable predictions of drug-specific personalized resistances to a matched antibiotic treatment based on an expected resistance profile (Yelin et al., 2019). Further, the concepts of precision farming have modified the notion of antibiotics in agriculture as it provides for the more targeted and responsible application of antibiotics to combat antibiotic resistance (Bothe, 2024).

Table 2: Impact on Antibiotic Prescribing Practices.

Aspect	Description	References
Improved Accuracy	Rapid pathogen identification reduces diagnostic uncertainty	Timbrook et al., 2017

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Enhanced Antimicrobial Stewardship	Enables early de-escalation of broad-spectrum antibiotics	MacVane & Nolte, 2016
Resistance Prevention	Reduces emergence of AMR by targeted therapy	Lipsitch & Siber, 2016

Antimicrobial Stewardship:

It leads to more judicious use of antibiotics through rapid molecular diagnostics when done in conjunction with antimicrobial stewardship programs (ASPs). More rapid organism identification and the consequential antimicrobial resistance ensure that a patient receives effective therapy sooner. This may lead to an overall decline in the need for broad-spectrum antibiotics. Studies have indicated that the best value of point-of-care diagnostics is achieved when combined with immediate ASP intervention, rather than merely reporting the results (MacVane & Nolte, 2016). Point-of-care diagnostic testing can also reduce the abuse of antibiotics leading to AMR (Schneider et al., 2020). Qualitative research has discovered that general practitioners are excited to use point-of-care tests that would differentiate between viral and bacterial infection, since it would enable them to not prescribe antibiotics when patients would not be benefited (Schneider et al., 2020). A complementary approach to reduce the overuse of antibiotics may be in diagnostic stewardship, or the choice of ordering tests (Woods-Hill et al., 2022). By avoiding unnecessary tests which will probably yield false-positive results, diagnostic stewardship may also prevent the "nudge" for an action in this case antibiotic treatment (Vaughn et al., 2023). Rapid and precise microbiological diagnostics, for example, nanopore metagenomics, can now provide directed treatments and limit unnecessary broad-spectrum antibiotics prescriptions (Charalampous et al., 2019). These molecular methods can identify the pathogens and their antibiotic-resistant profiles in a few hours with early targeted therapy and support on antibiotic stewardship (Charalampous et al., 2019). For example, in the case of deficit of more definitive diagnostic apparatus, the barriers to optimally prescribing antibiotics can be overcome by the rapid diagnostic methods (Pulia et al., 2022). Diagnostic stewardship interventions, which use the capabilities of rapid, multi-pathogen detection panels, have the potential to decrease the time to provision of pathogen-directed treatment, thereby possibly reducing unnecessary or broad-spectrum antibiotic use (Markussen, 2023). All the references above support the view that real-time diagnostics, when applied in conjunction with antimicrobial stewardship programs and diagnostic stewardship strategies, contribute to reducing overuse and misuse of antibiotics through more rational and targeted use of the drugs in question (MacVane & Nolte, 2016; Woods-Hill et al., 2022; Schneider et al., 2020; Charalampous et al., 2019; Markussen, 2023).

Resistance Prevention:

Antimicrobial stewardship is identified as an essential intervention for reducing inappropriate or unnecessary antibiotic use and AMR (Corcione et al., 2021; Cannella, 2010). ASPs encourage practices such as the early de-escalation of empiric antimicrobial treatment, narrowing the spectrum or reducing the number of antimicrobial agents used once culture results are available (Corcione et al., 2021). Studies have revealed that ASPs will even reduce the development of resistance together with reducing the healthcare cost of a country (Cannella, 2010). Infection control and antimicrobial stewardship must be effective to reduce the arising and spread of AMR

(Ketcherside et al., 2020). However, robust ICASPs in the implementation by LMICs face challenges such as poor laboratory, staff, and diagnostics tools training and lack of data on antimicrobial usage and resistance (Ketcherside et al., 2020). Informatics infrastructure is absent among LMICs in places they do not have any kind of established infrastructure; mobile cellular technologies and smartphones can be used for supporting ICASPs, and antimicrobial stewardship (Ketcherside et al., 2020). Promising findings related to antimicrobial stewardship and infection control improvement of the Colombian hospitals have also been reported in the case of cloud-based mobile applications (Ketcherside et al., 2020). Automated digital broth microdilution-based platforms for susceptibility testing enhance precision and convenience, which can direct appropriate therapy and restraint the emergence and spread of resistance (Smith, 2016). There has been the development of new microneedle-based, electrochemical sensing technologies that potentially may find clinical applications in antimicrobial therapeutic monitoring and optimize antimicrobial agent dosing to increase patient outcomes, which consequently limit the development of resistance; (Rawson et al., 2017). Vaccines can help to solve the problem of AMR by decreasing the demand for antimicrobial use and the overall number of cases, which means decreasing the selective pressure for the development of resistance (Lipsitch & Siber, 2016). Vaccines against pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* will also allow for narrower-spectrum antibiotics for empirical therapy (Lipsitch & Siber, 2016). The spread of antimicrobial resistance genes and resistant bacteria are associated with complex interactions of socioeconomic and environmental determinants (Vikesland et al., 2019). Efforts to monitor and intervene at the environmental source, such as in wastewater, can progress towards the diminution of the worldwide spread of AMR (Risely, 2024).

6. Case Studies and Clinical Evidence

Real-time pathogen detection technologies have been integrated in the emergency departments of many healthcare facilities to better manage infectious diseases and provide diagnoses. Among these technologies, mNGS has proven several benefits compared to the conventional culture-based methods (Wu et al., 2020). This technology has also indicated a sensitivity more so than traditional culture methods for quite many rare, novel or barely detectable pathogens; indeed, it has the potential for discovering all types of pathogens simultaneously with a single test to determine the range from bacteria through viruses and parasites (Qian, 2023; Zeng et al., 2021). It is hereby recommended that mNGS be applied when there is still time, especially when multiple infections are suspected to take place (Qian, 2023). In its implementation in EDs, some researches have actually proven that mNGS can successfully diagnose many of the infectious conditions. For example, in a case report where eschar was not present, mNGS was applied to identify the causative pathogen of scrub typhus (Wu et al., 2020). Another case report explained how mNGS was used in diagnosing *Klebsiella pneumoniae* invasive liver abscess syndrome with purulent meningitis (Zeng et al., 2021).

The application of mNGS within ED has also been demonstrated to offer utility in the management of acute gastroenteritis. A multicenter, prospective study demonstrated that,

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compared with clinician-selected diagnostic testing, multiplex molecular diagnostics, such as mNGS, reduced the possibility of return visits to healthcare by 21% (Pavia et al., 2023). Further, these diagnostics significantly improved detection of potential pathogens, including treatable pathogens and pathogens for withholding of empiric antibiotics, Pavia et al., 2023). The implementation of mNGS in the ED has been shown to increase the detection rate of the causative microorganism in patients with bacteremia and can therefore allow for targeted antibiotic therapy and subsequent de-escalation of broad-spectrum empiric antimicrobial therapy. Most of the pathogens that were also recovered were gram-negative bacteria in a recent study, hence of concern in light of the growing resistance of the organisms against commonly used antibiotics (Rothe et al., 2019). Although the overall positive rate of mNGS in the ED setting was reported to be lower compared to other departments (Qian, 2023), the technology has been recommended as a critical supplement to current conventional culture methods, especially in complicated cases of infectious conditions (Qian et al., 2021). Zeng et al., 2021, have also suggested further validation of the workflows, reduced costs, and easier criteria for interpretation to ensure routine clinical adoption for the successful implementation of mNGS in EDs. Besides mNGS, the successful implementation of real-time pathogen detection technologies in the diagnosis of infectious gastroenteritis in EDs has also included multiplex PCR. These technologies have improved the rate of pathogen recovery and helped detect pathogens beyond the countermeasures. This is witnessed in Montasser et al. (2022).

7. Challenges and Limitations

Integration and Interoperability

Integration with current health information systems usually becomes challenging for most of the new technologies. Great barriers might be multiple sources of data integration, security of data, and the protection of privacy. Other barriers might include a difference between the model of technology and clinical workflow (Greenhalgh et al., 2017; Pallin et al., 2010). Other barriers include inadequate strong IT infrastructure and difficulty in transferring the technology to the ED environment as required (Pallin et al., 2010; Østervang et al., 2022). Nonacceptance by healthcare providers and aversion to change also hinders the integration of digital technologies. It can cause disruption in the continuity of clinical practices and demand a large amount of training and learning time before application (Greenhalgh et al., 2017; Shirazi et al., 2022). Lack of management support, especially no felt value or perceived demand for technology, like in Shirazi et al. (2022), is another challenge. Complexity and heterogeneity in the implementation process across healthcare setups are challenges. Differences in delivery care models, institutional preferences, and availability of resources tend to cause inconsistencies in their adaptation or implementation processes (Pallin et al., 2010; Rasmussen et al., 2021). High workload with strict time boundaries in the environment of EDs may also create hindrance toward effective utilization of new technology (Greenhalgh et al., 2017; Shirazi et al., 2022). Considering the time barrier, adequate time may not be taken by the service providers in learning about these new systems and integrating those in day-to-day flows (Shirazi et al., 2022). Finally, the cost of purchasing and maintaining these technologies can become a major hindrance especially in resource-limited

health care settings (Pallin et al., 2010; Miller et al., 2021). Sustaining and scaling up such technologies in the long run becomes another issue (Østervang et al., 2022).

Table 3: Challenges and Solutions in Implementing Pathogen Detection Technologies.

Challenge	Description	Proposed Solution	References
High implementation costs	Equipment and maintenance expenses	Subsidies and collaborative funding	Pallin et al., 2010
Integration difficulties	Compatibility with existing systems	Interoperable software development	Greenhalgh et al., 2017
Training deficits	Lack of expertise in using new technologies	Comprehensive training programs	Konttila et al., 2018

According to the references, cost, training, and compatibility with the current hospital systems are some of the critical elements in the adoption of new technologies within healthcare settings. The main cost factor in adopting new technologies includes setting up new infrastructures and software with high maintenance. Applying cloud-based platforms diminish the costs involved in building an operating in-house database" says Jin. According to Nirantharakumar, the value needs to be compared overall for it not to be considered cost effective (2012). Also, it considers the "training of health care workforce" that involves healthcare staff (Konttila, et al, 2018; Ivarsson et al, 2016; McAlearney et al, 2012). It then requires that staff be sufficiently trained and supported to use the systems effectively to implement new technologies (Konttila et al., 2018; Ivarsson et al., 2016; McAlearney et al., 2012). That ranges from technical training, through education on how this technology can fit into workloads and clinical practices (McAlearney et al., 2012). The adoption of the technology may lead to poor uptake and suboptimal use if there is insufficient training (Nirantharakumar et al., 2012). Integration with existing systems within hospitals can also enhance the adoption of new technologies in the health sector (Jin, 2016; Wang & Wang, 2019; Lo et al., 2015). Integration with EHRs, HIS, and other clinical workflows needs to be seamless so that the data can be shared, information can be collaborated on, and efficient clinical care is provided (Jin, 2016; Wang & Wang, 2019; Lo et al., 2015). Failure in integration would raise difficulties and frustration for the health providers and thus resistance towards the new technology would emerge (Lo et al., 2015). Furthermore, the organizational and cultural context of the healthcare setting is also influential in technology adoption (Konttila et al., 2018; Knudsen et al., 2022). The aspects that include team climate, leadership support, and overall readiness for change within the organization determine how easily new technologies are accepted and integrated (Konttila et al., 2018; Knudsen et al., 2022). Engaging healthcare professionals in the implementation process and ensuring their concerns are addressed can enhance successful adoption (Knudsen et al., 2022).

8. Advances in Real-Time Detection Technologies

Automation and Integration:

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The following is one of the major developments made to greatly enhance the efficiency and usability of pathogen detection tools. One major development is nanopore sequencing technology, such as the MinION platform, that allows the direct and rapid identification of bacteria and viruses in complex DNA samples (Kilianski et al., 2015). These technologies therefore bypass culturing and amplification steps that otherwise may delay the pathogen-detection process. The application of surface plasmon resonance biosensors offer another critical innovation that should enable the miniaturization of devices to detect pathogens with improved detection throughput with lower operating costs. Additionally, SPR-based sensing technologies have been found to exhibit a potential in replacing some traditional diagnostic methodologies for detecting pathogens rapidly at high sensitivities (Park et al., 2022). Another notable innovation is the microfluidic systems as a strong point-of-care pathogen detection tool. The automated devices, with their microchannels, manipulate and analyze tiny volumes of liquid to identify many pathogens such as bacteria, viruses, fungi, and parasites in quick time and with good accuracy (Nasseri et al., 2018). Molecular tools like quantitative PCR also evolved continuously, making pathogen detection more efficient. However, it must also be critically important from time to time to validate those assays to ensure that, at minimum, they can support at least minimum levels of functionality and strictly adhere to MIQE guidelines (Geraci-Yee et al., 2022). Techniques like droplet microfluidics for the analysis of single cells have turned out to be an indispensable component in the development of tools and procedures for detecting pathogens by making it possible to isolate individual pathogenic microbes for detailed analysis (Wang, 2023). This methodology therefore enables an integrated perspective over microbial interactions that are rather complicated.

The means for post-harvest disease of fresh produce has also enhanced owing to improved methods in pathogen detection and control strategies (Youssef et al., 2023). Of course, more studies will be conducted before more environmentally friendly, yet viable control methods come along but these have significantly advanced efficiency and usability in tool technology for pathogen detection. Next-generation sequencing technology has revolutionized pathogen detection methodologies, with higher sensitivity and the possibility of detecting a full genome sequence of pathogens including unknown species (Li, 2018). Such an outstanding advantage of NGS explains its increased application in clinical diagnostics and pathogen identification. Modern infectious pathogen detection with the development of strong bioanalytical devices and biosensors also advanced further with the integration of contemporary communication systems like the IoT and ML, which expand the scope of their application (Ngashangva et al., 2022). Smart technological platforms that rapidly identify, classify, and assess the concentration of pathogenic bacteria can assist in making decisions regarding their removal and treatment strategies. Nanotechnology has also revolutionized the detection tools of pathogen, increasing the efficacy and usability of pathogen detection tools. Nanomaterials have special features, including higher surface permeability, a greater surface-to-volume ratio, and reactivity, making them suitable for designing biosensors that are highly sensitive but cost-effective in detecting microbial pathogens in food (Kumar et al., 2020).

Nanotechnology and Microfluidics:

The role of nanoscale devices and lab-on-a-chip (LoC) platforms in real-time diagnostics is multidimensional. It has been well studied in the literature. Both technologies have immense advantages through which rapid, sensitive, and cost-effective point-of-care diagnostics are feasible. However, this is essential to the management of many diseases that are life-threatening (Dak et al., 2016). Nanodimensional devices such as nanoparticles, nanowires, and carbon nanotubes provide intrinsic properties for the application of nanoscale device-based diagnostics. Functional nanomaterial-based lab-on-a-chip microfluidic devices have presented enhanced detection limits, improved signal-to-noise ratios, and greater capture efficiencies coupled with innovative strategies for configuring the sensing architectures (Vaishampayan et al., 2023).

Nanoscale lab-on-a-chip devices can be integrated into the lab-on-a-chip platform, which is a miniaturized, automated, and integrated system that can carry out an entire set of biomedical assays (Dak et al., 2016; Ali, 2020). The advantages of microfluidics, optics, electronics, and biosensors help make LoC platforms cost-effective, high-throughput, and sensitive for point-of-care diagnostics (Ali, 2020; Sonntag et al., 2016). Such platforms can have, on one chip, many analyses, including biochemical operations, chemical synthesis, and DNA sequencing, which otherwise would require significant time and resources in a traditional laboratory setting (Gupta et al., 2016).

LoC technology miniaturized and rapidly prototyped to make it very useful in various applications from environmental analysis, medical diagnostics, and pharmaceutical testing (Sonntag et al., 2016). The major advantages offered by LoC platforms are that they are able to make real-time diagnostics. This is generally because LoC platforms combine several entirely different components, for example microfluidic channels, sensors and detection systems in one location for the objective of speedily continuously monitoring physiological signals and biomarkers (Zheng et al., 2016; Nguyen et al., 2021; Huang et al., 2023). This is very important, particularly for early diagnosis and the treatment of acute or chronic diseases, which would have been curable if diagnosed and properly personalized care had been provided (Zheng et al., 2016). Additionally, the applications of real-time diagnostics have been increased further with the integration of LoC platforms with advanced technologies, such as smartphones and wearable devices (Hernández-Neuta et al., 2018). LoC devices can be made smart through smartphones that make readout platforms portable, flexible, and very connected, to democratize evidence-based health care (Hernández-Neuta et al., 2018). Wearable sensors with inclusion of technologies such as triboelectric nanogenerators can even be integrated with LoC platforms to continuously monitor important physiologically relevant parameters, such as motion, sleep states, and ion concentrations, so that early disease detection becomes possible and customized health care is provided (Nguyen et al., 2021; Huang et al., 2023; Li et al., 2021). Like that experienced with the COVID-19 pandemic, the speed by which microfluidic devices that are to be used for SARS-CoV-2 detection were developed demonstrates in full the agility and adaptability of LoC platforms for any new and emerging health issues. (Berkenbrock et al., 2020). Through the utilization of recent breakthroughs in our science of the virus and possible availability of biological ligands, researchers have made it possible to rapidly develop miniaturized, portable LoC-based detectors for COVID-19 (Berkenbrock et al., 2020).

Sajidah Taqi Baqer Alshakhs, Radiah Hussain Ali Alsafar, Ali Hussain Alhumud, Ahmed Mohammed Aljubarah, Batool Sadiq Taher Aldahneen, Abdullatif Hussien Khadem AlAbdullatif, Mohammed Ali Ahmed Alkhawaja, Ali Hussain Alhaddad, Zahraa Jafar Alsultan, Iqbal Ibrahim AlAli, Farhan Hameed Albaiji, Aljwharah Abood Nami Aljuaid, Mohammed Awadh S Alharbi, Maha Mubarak Mesfer Aldawsari, Hind Almadi Khalaf Alrowli

AI and Machine Learning:

AI-powered analytics may dramatically enhance the precision of detection and decrease false positives/negatives in the technology used for real-time pathogen detection. Using the capabilities of machine learning and deep learning algorithms, researchers have come up with advanced analytical frameworks which can process large chunks of sensor data and extract the high-precision patterns that indicate the presence of pathogens ("RPA and AI-Driven Predictive Analytics in Banking for Fraud Detection", 2022). One such strategy is supervised learning algorithms, trained models classifying pathogenic variants with high reliability. For example, Al-Jarf et al. (2022) reported developing gene-specific predictive models while assessing the functional consequences of missense mutations in BRCA1 and BRCA2 genes and improving the classification of pathogenic variants. This demonstrates that AI-based analytics have tremendous potential to further the detection capabilities of pathogens to very great accuracy, in this case genetic marker-based. Toward detection of pathogens, Bradford et al. (2023) developed an optimal pipeline on detecting Salmonella sequences through shotgun metagenomics. Researchers have rigorously tested the pipeline on mock communities of known pathogen provenance, thus evaluating the correctness of the system and providing insights into how false positives as well as false negatives might be mitigated. This article well explains why any AI-driven analytical framework applied in pathogen detection must be adequately tested and validated. Doppler radar sensors are another kind of sensor technology that could be coupled with AI-driven analytics to make pathogen detection systems more precise. Al-Okby & Thurow (2021) presented a fall detection system using data from motion-tracking sensors and Doppler radar for high accuracy of the system with minimum false-positive and false-negative errors. This multi-modal approach suggests that AI-driven analytics with different sources of data enhance the performance of real-time pathogen detection technologies. The use of AI-powered analytics is not just reserved for genetic or sensor-based methods. Tansarli & Chapin, in 2020 conducted a systematic review with a meta-analysis on the accuracy of the diagnostic test the BioFire® FilmArray® meningitis/encephalitis panel, a multiplex PCR assay. This work gave an issue with false positives and false negatives, implying that even the most developed analytical approaches would be required to help these issues. Sharma (2024) employed optical sensing in the real-time detection of foodborne pathogens, such as Escherichia coli and Salmonella enterica, from fresh produce by using machine learning algorithms.

Detection accuracies in the research report were at 95%, significantly more than traditional methods that are less accurate. This exemplifies the potential of AI-driven analytics to further increase precision and speed of pathogen detection in several applications in food safety. Though the development of AI-driven analytical frameworks for pathogen detection appears promising, this also calls for consideration of its potential biases and limitations. Alirezaie et al. 2018 argued that type I circularity, a phenomenon discussed in detail in relation to supervised machine learning, eventually leads to overfitting and hence to models having a lack of reliability must be avoided. This therefore calls for rigorous validation and testing of AI-driven analytical tools to ascertain their robustness and generalizability. Lastly, Brost et al. (2018) pointed out that correction of some observational errors such as false-negative and false-positive errors needs to be addressed using molecular techniques used in detecting microorganisms, macroorganisms, or

infectious agents. This helps in improving the reliability of pathogen detection systems with occupancy models to account for such errors. (Brost et al. 2018)

9. Future Directions and Innovations

The development of novel protocols, using smart third-generation sequencing technologies, has made it feasible to use real-time pathogen detection. Such technologies are highly capable of quick diagnosis, surveillance, and even real-time tracking of infectious diseases and are now enabling the possibility of a global, real-time digital pathogen surveillance system (Tümmler, 2020). Real-time colorimetric quantitative molecular detection applied on smartphone-based diagnostic platforms is a recent trend. LAMP detects and measures the number of nucleic acids present through amplification for the detection, which would be more affordable and reachable at the point-of-care (POC) compared with the traditional fluorescence-based method of detection (Yin et al., 2020). Universal photonic biosensors for real-time detection of emerging pathogens Another trend is that universal photonic biosensors for real-time detection of emerging pathogens are developed. These types of biosensors apply the probe-cleavage detection principle and can provide rapid novel ultra-sensitive platforms without requiring the receptors to be target-specific (Blevins et al., 2021). Another area of application where CRISPR-Cas12a is seen to have potential is as an ultrasensitive and visually detecting tool for pathogens such as SARS-CoV-2. It will be highly useful to detect pathogens within a very short time frame using the ultrasensitive all-in-one dual CRISPR-Cas12a assay in a POC-friendly manner. ". Other growing trends include real-time loop-mediated isothermal amplification, or in brief, real-time LAMP, with its benefits for rapid and sensitive detection of pathogens, even at a low level of DNA infection (Ding et al., 2020). This addition of loop primers has improved the amplification efficiency, with the advantage of having the results be visualized directly (Cai et al., 2018).

Handheld, smartphone-operated devices that run real-time colorimetric LAMP-based diagnostic devices for infectious diseases have emerged. For instance, there is the SMART-LAMP device. The SMART-LAMP takes advantage of the availability of Bluetooth connectivity and mixes available to be used in place. Thus, these would better apply in POC settings (Diego et al., 2022). Apart from the molecular methods, there are also rapid immunochromatographic test kits that detect mastitis-causing streptococci in cattle. It is relatively easier and faster than complicated methods such as real-time qPCR (TSUGAMI, 2024). The trend is for the use of NGS technologies, such as nanopore sequencing, to identify and characterize new and emerging viral pathogens rapidly in veterinary and public health. Real-time surveillance and the detection of pathogens are accomplished by the nanopore technology through nanopore sequencing that does not rely on prior knowledge nor specific primers (Neujahr, 2024; Er et al., 2021). Besides that, there is a trend for multiplex real-time PCR assays which are used in the detection of several pathogens that are causing diarrhea like *Cryptosporidium*, *Giardia*, and *Entamoeba*. Such multiplex assays have shown benefits for being more efficient with greater adaptation toward individual diagnosis needs (Paulos et al., 2019; Sánchez et al., 2022). Finally, the adoption of participatory approaches, which include crowdsourced data such as iNaturalist and eTick.ca,

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marks a growing trend in the real-time surveillance of disease-carrying vectors and vector-borne pathogens (Rilkoff, 2024).

10. Conclusion

Introducing real-time pathogen detection technologies represents an important milestone in emergency medicine and has addressed long-standing challenges regarding the diagnosis of infections and the management of antibiotic stewardship. The list includes PCR and CRISPR-Cas systems, POC testing, and NGS. This list identifies pathogens along with their respective antibiotic resistance profiles with the accuracy that would be possible in terms of speed and precision. Accuracy results in reduced antibiotic usage, regulated antimicrobial resistance, and increased therapeutic interventions. Analytics that depend on AI improves diagnostic accuracy and saves time by analyzing complex data to make appropriate clinical decisions on time. Real-time pathogen detection in ED workflows has brought immense benefit in terms of a reduction in turnaround times in diagnostics, improved patient outcomes, and enhanced stewardship of antimicrobial therapy. However, there are challenges that face the implementation, such as costs, strict clinical validation required, and their integration into the existing health care system. This is sure to be an area that is overcome by concerted efforts of the healthcare providers, researchers, and policymakers. The promise of future innovation in smartphone-based diagnostics or biosensor-based platforms gives exciting promise toward the democratization of technology, even into poor-resource settings. Transforming global health strategies, healthcare systems would be better positioned with more surveillance and response capabilities of healthcare systems through real-time detection tools, AI, and IoT-enabled platforms. Real-time detection of pathogens is one major frontier of emergency medicine for the sake of more accurate, speedy, and equitable delivery. Their development and eventual integration hold the promise of revolutionizing the diagnostic landscape with the goal of fighting the growing global threat of antimicrobial resistance.

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Author contributions

The initial text was written by the corresponding author, although all authors made substantial contributions by gathering data and doing a literature search. Each author agreed to take responsibility for every portion of the work, participated in the manuscript's critical revision, and approved the final draft.

Conflict of Interest

The authors declare they don't have any conflict of interest.

Ethical Approval

Not Applicable

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