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EVALUATION OF NEW TECHNOLOGIES FOR RAPID DIAGNOSTIC TESTING IN THE LABORATORY

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Abstract

Assessing novel technologies for expeditious diagnostic testing inside laboratory settings is of paramount importance in effectively tackling the urgent issues surrounding antibiotic misuse and antimicrobial resistance (AMR). The existing condition of medical facilities is characterized by a deficiency in a comprehensive and efficient diagnostic system capable of accurately identifying the etiological agent, distinguishing between viral and microbial illnesses, detecting antibiotic resistance, and providing guidance for suitable antimicrobial interventions. The primary objective of this research is to evaluate the progress made in diagnostic technologies and comprehend their possible implications in tackling these difficulties. This research investigates a range of technical developments in the field of quick diagnostic testing. These advancements include nucleic acid synthesis methods, electrochemical techniques, microarrays, micro- and nanoparticles, as well as mass spectrometry applications. These technological advancements possess the capacity to greatly enhance the efficiency and precision of infectious illness diagnosis, therefore empowering healthcare practitioners to make treatment choices based on empirical data and mitigate the abuse of antibiotics. Significant endeavors are now underway to augment antibacterial susceptibility and microbial detection methodologies. Conventional approaches, while efficient, may be laborious, sometimes requiring many days for outcomes. The research emphasizes the need for expeditious diagnostic tools that provide prompt and precise outcomes, especially in outpatient environments where such tests are often inaccessible. In summary, the assessment of novel technologies for expeditious diagnostic testing inside laboratory settings has encouraging prospects for tackling the obstacles presented by antibiotic misuse and AMR. The possibility for revolutionizing infectious disease treatment, reducing antibiotic abuse, and efficiently managing AMR exists with the implementation of these developments. Nevertheless, more investigation and regulatory authorizations are essential to guarantee the extensive use of these technologies in medical facilities around the globe.

Keywords: rapid diagnostic testing, laboratory, antibiotic abuse, antimicrobial resistance, medical centers.

1. Introduction

The current state of medical centers lacks a comprehensive and efficient diagnostic system that can effectively address the issue of antibiotic abuse. It is crucial to determine the causative pathogen and distinguish among viral and microbial infections, as well as detect the antibiotic resistances in microorganisms and choose the appropriate antimicrobial medication for treatment. Consequently, the superfluous use of antibiotics might be reduced and the dissemination of antibiotic resistance more effectively managed. The World Health Organization (WHO) has identified antimicrobial resistance (AMR) as the most significant worldwide health risk in the 21st century, requiring immediate action. The rise of antimicrobial resistance (AMR) has rendered common illnesses more untreatable (1).

According to the United Nations summit on antimicrobial susceptibility in 2016, the annual mortality rate due to drug-resistant diseases exceeds 700,000 individuals, with projections indicating a potential increase to 10 million by the year 2050 (2). Based on existing knowledge, antibiotic-resistant microorganisms claim the lives of around 33,000 individuals annually in EU and EEA nations. According to Cassini et al. (3), they are responsible for around 900,000 disability-adjusted years. The proximity of diagnostics to medical professionals and subsequently to patients would result in a fundamental change in the approach of treating infectious illnesses in outpatient clinics, transitioning from experimental to evidence-based therapies. Swift diagnostics are required for both the detection of pathogens and the testing of resistance.

Certain organisms in certain geographic regions may exhibit a very high frequency of antimicrobial resistance (AMR). Based on the prevailing guidelines for antimicrobial susceptibility tests (AST), it is recommended to employ cultured isolates for the purpose of evaluating the impact of antimicrobial medications. This is necessary because the composition of the specimen substrate (blood, urine, mucosal) and the abundance and distribution of various microbial species might significantly change in polymicrobial specimens. The classification of the identified microorganism as either pathogenic or commensal remains uncertain. Although there have been notable advancements in diagnostic tools in recent times, the majority of patients with infectious disorders continue to get treatment based on intuition, resulting in excessive usage of antibiotics (4,5).

According to the Agency for Disease Control Management (6), thirty percent of prescribed antibiotics in Western nations are deemed unneeded or inappropriate. Inpatients benefit greatly from current diagnostic techniques, but, these tests are frequently unavailable in outpatient clinics. For conventional growth-based antimicrobial susceptibility testing (AST), many cultivation cycles are necessary: enriching cultivations (such as blood cultures) to augment the bacterial population, plate cultivations to acquire uncontaminated cultures, and ultimately AST for liquid or plate samples employing different antibiotic concentrations. In microbiology labs, the use of EUCAST-accepted breakpoint values is employed to determine the susceptibility or resistance of microorganisms to the antibiotic under investigation. In order to adhere to EUCAST criteria, the disk diffusion technique or other calibrated methods are used. In all, AST may need many days.

Numerous scholarly articles have extensively examined the topic of rapid molecular diagnostics (7,8,9,10). The authors provide an overview of advancements in Nucleic Acid Synthesis Techniques (NAAT), electrochemical techniques, microarrays, micro- and nanoparticles, and mass spectrometry applications. However, they also highlight the limited number of molecular approaches that have obtained clearance from the Food and Drug Administration (FDA).

The article titled "Tuberculosis Diagnostics Technology Landscape" by David Boyle is a valuable resource for readers. While it does not specifically address AST, it provides a comprehensive overview of novel molecular diagnostic technologies that are accessible through standard microscopy stations, with a particular emphasis on their availability in developing nations (11). Congress presentations, talks, and webinars provide the most current and succinct progress compendia in the area of AST (12).

New methods in AST have been developed as a result of advancements in electronic devices, biosensor approaches, optical technology, microfluidics, hybridization innovations, and DNA replication techniques. Regrettably, the scientific literature pertaining to these technologies lacks a comprehensive connection between the results and the practical requirements in the field of POCT. In order to establish a microbiology laboratory, it is necessary to provide sufficient time and resources for the enhancement of cultures and the subsequent creation of pure cultures. The consideration of sample interventions is frequently inadequate. Furthermore, these needs might readily obscure the overall expenses of AST. The implementation of novel molecular techniques for AST is very sluggish because to these factors (13). The standards of care pertaining to antibiotic prescription exhibit a high degree of consistency across several European nations and the United States. These criteria adhere to evidence-based identification and AST, if feasible.

Urinary and respiratory infections together constitute a substantial proportion of acute infections. Efficient and precise detection of these infections within outpatient settings has the potential to effectively mitigate the transmission of antimicrobial resistance (AMR) bacteria, enabling early separation of the transmitter and appropriate treatment. Swift diagnosis would further enable the expeditious removal of superfluous patient isolation, resulting in cost and resource savings. Nevertheless, the advantages of technology advancements are contingent upon the physicians' implementation of structured communication and analysis of the findings, as well as the reasonableness of the associated costs (10).

Considerable efforts have been allocated to the field of urgent investigations for septicaemia (14,15). Emerging techniques, including T2MR (T2Biosystems, USA), have shown the capability to promptly identify molecular targets based on clinical specimens. This advancement facilitates the swift identification of pathogens and the detection of resistance components. Nevertheless, the use of growth-based AST for blood necessitates a rather elevated bacterial count for enrichment cultures and a microbiology laboratory that is well equipped. The correlation between advancements in blood tests and antimicrobial stewardship in hospital settings may not be directly proportional.

2. Advancements in Antimicrobial susceptibility and Microbial Detection Techniques

Bacterial resistance to antibiotics may be acquired via many processes. The degradation or chemical modification of antibiotics may occur by several processes, including as nucleotidylation, acetylation, ADP-ribosylation, phosphorylation, glycosylation, and mono-oxygenation. The prevention of drug intake or the enhancement of efflux may be implemented. Resistance mechanisms may be achieved by altering the production of the cell wall. Even minor alterations in the target molecule, such as one modification in the ribosomal protein, have the potential to diminish the effectiveness of the antibiotic. AST is complicated by the vast array of antimicrobials and mechanisms of resistance.

Genotypic approaches, which rely on nucleic acids, may only detect resistances that are specifically targeted, and resistance gene sequences that may be identified may not necessarily originate from the real pathogenic organism. As to the standards set by EUCAST and CLSI, accurate diagnoses for antibiotic resistance need phenotypic testing, which involves conducting a laboratory experiment to determine whether the bacterium exhibits growth in the presence of the medication. These strategies are effective irrespective of the mechanism of resistance and provide solutions to practical inquiries on the most effective antibiotic and the appropriate dosage for treatment. Traditional antimicrobial susceptibility testing (AST) methods, including broth microdilution, disk dispersion, gradient examinations, agar dilution, and breakpoint examinations, include the continual introduction of an isolate of bacteria to a specific group of antimicrobial agents, followed by the visual observation of growth of bacteria. Enhancing the responsiveness and efficiency of optical devices may be achieved by the use of modern optoelectronic structures, fiber optics, microfluidics, and indicator dyes that are responsive to redox-state or pH (15).

Numerous commercial technologies have been developed to enhance and partially automate the process of monitoring AST cultures. Automated turbidity measuring for multiwell liquid cultures is conducted by systems such as Vitek and Microscan. The use of a redox indicator in the BD Phoenix system™ serves to augment the identification of organism development. The ID turnaround times for these systems are as fast as 4 hours, whereas the susceptibility testing turnaround times range from 6 to 8 hours (16). The Alfed 60 AST™ system, which is CE-marked and manufactured by Alifax in Italy, employs advanced laser-light dispersion innovation to identify the presence of bacteria in a liquid culture broth. This system immediately offers antimicrobial susceptibility data from positive culture containers within a time frame of 4 to 6 hours.

These techniques that rely on broth dilution use pre-made AST cassettes or cards that have positive controls and wells containing antibiotics at progressively higher doses. Their comprehensive databases enable them to conduct continuous growth monitoring and assess minimum inhibitory concentration (MIC) trends for a wide range of species.

Pathogen detection (ID) often serves as a first stage in the process of AST. Microscopy as well as Gram-staining are often used for blood samples because to the relatively narrow range of antibiotic susceptibility and lower occurrence of multidrug resistance in Gram-positive bacteria.

In AST, the standard procedure involves culturing clinical samples to acquire pure isolates. Next, the process of identification is conducted using a MALDI-TOF mass spectrometer, if one is accessible. Subsequently, the determination of AST and MIC is conducted in accordance with the EUCAST or CLSI guidelines. The whole of this sequence necessitates a span of many days. Advanced microbiology labs equipped with mass spectrometry equipment are not often seen in standard healthcare settings. They may only have access to immuno-chromatographic strip tests, also known as lateral flow tests (LF or "dip-sticks"), which are used to identify viruses such as influenza as well as bacterial pathogens that cause sexually transmitted illnesses.

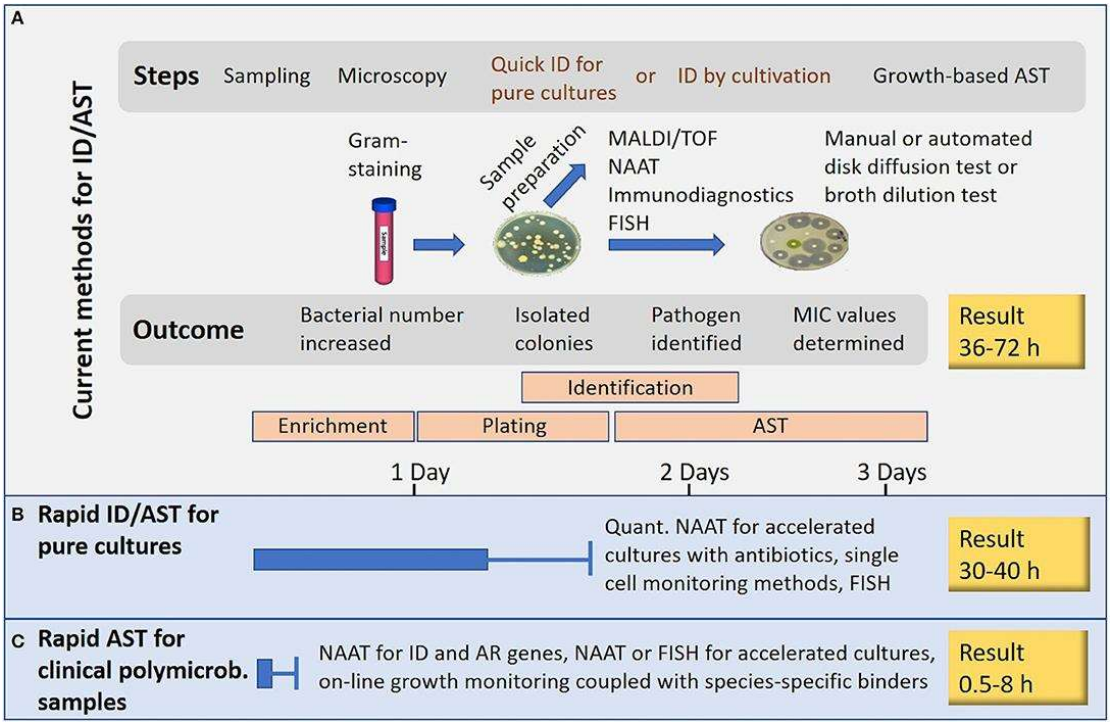
Rapid identification may effectively limit the range of antibiotics that can be searched. In clinical labs, mass spectrometry has emerged as a flexible and indispensable tool. Bacterial identification is often used promptly upon the availability of isolated colonies. The acquisition of a biochemical signature of bacteria may be achieved by concurrently measuring several metabolites. The Matrix-assisted laser desorption/ionization period of flight (MALDI-TOF) technique involves the use of laser energy to induce evaporation of the sample bonded to the matrix. Subsequently, the sample is promptly subjected to analysis. According to Maxson et al. (17), the use of frequent sampling in MALDI-TOF may provide semi-quantitative development information. Bruker Corp., a German company, has developed test kits, including the BT STAR-Carba Assay, which is designed for measuring antibiotic breakdown in AST.

The use of AST for blood cultures involves doing a brief culture on agar plates and then conducting susceptibility tests using VITEK AST cards. The selection of VITEK AST cards is based on MALDI-TOF analysis, as described by Idelevich et al. (18) and Mauri et al. (19). According to Ceyssens et al. (2017), the use of the MBT-ASTRA™ test in conjunction with the MALDI Biotyper for identification and antimicrobial susceptibility testing (AST) enables the identification of mycobacterial strains that exhibit resistance to rifampicin, isoniazid, linezolid, ethambutol, clarithromycin, and rifabutin. This method offers a one-week advantage over the conventional cultivation-based AST. The "Future technologies" section will show the integration of MS techniques with NAAT or microfluidics.

3. Contemporary Technologies for Swift AST

Several innovative techniques claim to execute AST within minutes or a few hours (1). These assertions often overlook the need of labor-intensive procedures, such as the cultivation of enriching cultures and the separation of pure cultures (Figure 1).

Figure 1. Quick AST (1).



In theory, the utilization of non-purified polymicrobial medical specimens is facilitated by methods that rely on NAAT, nucleic acid hybridization, or immunodiagnostics. An abbreviated culture using a predetermined amount of antibiotics, followed by NAAT (such as isothermal amplification), may uncover antibiotic resistance (AR) and perhaps provide an approximate estimation of the minimum inhibitory concentrations (MIC) for the antibiotics that are being evaluated. Many AST approaches that focus on quick development only do end-point analysis, while others depend on regular sampling from the culture chamber. Certain immunodiagnostic systems that are sensitive have the capability to provide real-time online growth monitoring. Experimental presentations of biosensor technologies that identify modifications to bacterial metabolism, motility, or heat generation have not yet been convincing. There is still a lack of fast, dependable, user-friendly, and cost-effective equipment that can be used for AST in outpatient clinics (20).

4. The Commercialization Of Future Technologies

It is anticipated that mass spectrometry would undergo significant integration with other advanced AST technologies, particularly in the field of septicaemia diagnoses. The MALDI-TOF Direct-On-Target Microdroplet Growing Assessment (DOT-MGA) involves the direct application of sample droplets, consisting of culture and antibiotics in a volume of 6 µL, onto disposable MS-target plates. These droplets are subsequently incubated for duration of 3-4 hours

and then subjected to analysis using MS (21). Currently, there are screening panels that can detect ESBL and AmpC β-lactamases in enterobacteria (22). In recent times, significant advancements in microfluidics, biosensor methods, isothermal amplification-based nanoanalysis techniques (NAAT), and immunodetection have emerged, offering a range of powerful devices that have the potential to revolutionize the field of AST.

The QuickMIC system developed by Gradientech integrates microfluidics and automated time-lapse photomicrography techniques to track the progression of inhibiting growth across a linear drug concentration. The method quantifies the variations in grayscale intensity in the photos resulting from the development of microcolonies and offers AST within a time frame of 2-5 hours (23). Nowadays, the system is pursuing CE-IVD certification and FDA authorization for AST in blood specimens.

The Q-Linea ASTar® utilizes time-lapse microscopy to continuously monitor blood cultures and prepare and monitor bacterial isolates in a completely automated manner. The system has the capability to handle 12 samples simultaneously and 50 samples per day, providing accurate minimum inhibitory concentration (MIC) values for a maximum of 48 antibiotics within a time frame of 6 hours. The system lacks ID functionality; however, it is capable of being linked to any ID platform.

The BacterioScan 216Dx system, manufactured in St. Louis, MO, USA, is capable of measuring both the optical density, also known as OD, and the scattered power of a sample by backward laser light scatter. This system has a sensitivity that is 10 to 100 times greater than that of conventional OD measurements. The present system has the capability to concurrently process 16 samples and execute real-time continuous development monitoring. Clinical urine samples exhibiting a concentration of E exceeding 104 cfu/ml may be detected for bacterial growth within a 3-hour timeframe. The bacteria mentioned in the study conducted by Bugrysheva et al. (24), Hayden et al. (25), and Montgomery et al. (26) include *Bacillus antracis*, *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Burkholderia pseudomallei*, and Yersinia pestis. The ongoing testing phase involves the evaluation of the BacterioScan 216R Rapid AST System.

The oCelloscope™ technology, developed by BioSense Solutions in Denmark, utilizes computerized time-lapse angled field imaging and image processing techniques to analyze ordinary 96-well plates. According to Fredborg et al. (27), the duration required for blood cultures that were positive to provide results varied between 1 and 4.2 hours. Nevertheless, the comprehensive evaluation of AST's overall performance remains inadequately assessed.

Alterations in the appearance or size of individual cells serve as indicators of growth much in advance of bacterial multiplication. Bright-field microscopy is used in single-cell morphological analysis (SCMA) to ascertain alterations caused by antibiotics in cells that are fixed on an agarose channel chip (28). The MultiPath™ technology, which is available for commercial use, utilizes non-magnified digital imaging techniques to identify biomolecules that have been labeled with antibody-coated fluorescent nanoparticles. Antibody-coated magnetic particles are employed for the purpose of binding to certain cells of interest and then attracting them towards

the surface of the camera. This process effectively eliminates any extraneous signals and enables a wash-free test for clinical samples. The system is capable of quantifying individual targets inside expansive regions and conducting growth monitoring for numerous target cells, hence facilitating the calculation of minimum inhibitory concentration (MIC) values. The MultiPath™ structure is now in the process of obtaining FDA approval.

ArcDia Ltd. (Finland) has commercialized the Two-Photon eXcitation fluoroscopy technique (TPX), which enables the immunodetection of biological molecules in tiny reaction volumes without the need for separation (29). The mariPOC® testing method was designed with the purpose of efficiently identifying pathogens. Polystyrene microparticles are used as solid carriers to construct immunocomplexes. These immunocomplexes consist of three components: a monoclonal antibody, an antigen, and a tagged monoclonal antibody. The concentration of the analyte is directly proportional to the size of the microspheres. Two-photon illumination from the surface of each of the microspheres is used to measure the immunoassay fluorescence signal. The application of unpurified medical materials and the ability to monitor live cells in AST cultures online are facilitated by this technique. Currently, this technology is undergoing clinical investigation for AST indications.

Incorporating RNA detection, Nanostring Technologies Inc. integrates genotypic and phenotypic AST. The GoPhAST-R platform is used for the identification of mRNA expression patterns in bacteria subsequent to exposure to antibiotics. The method has the capability to be used immediately for favorable blood culture containers. Bhattacharyya et al. (30) integrate machine learning evaluation of transcriptional alterations with the identification of resistance genes.

According to Lonsdale et al. (31) and Lim et al. (32), colorimetric sensor sets offer a cost-effective approach for the detection of volatile organic substances (VOCs) that are linked to microbial metabolism. Small molecule sensor (SMS) arrays technology, developed by Specific Technologies Inc (USA), has been successfully commercialized. This technology exhibits reactivity with the metabolic products generated by bacteria throughout their development process. The Reveal-AST printable sensor array technology exhibits a colorimetric pattern in response to the volatiles released throughout the growing process. It also provides species identification with a 94% accuracy rate (33).

According to Schoepp et al. (34), the SlipChip/dLAMP system developed by Caltech effectively combines culture with semiquantitative smartphone-based visual monitoring of NA products. The forthcoming commercialization of this system by Talis Inc. (USA) will be expounded upon in further detail under the chapter titled "POCT-compatible technologies."

5. Challenges Associated with Miniaturization and Quick AST

Rapid AST may not provide data that accurately reflect the whole bacterial community in the diagnostic sampling because to the tiny sample size, which is often limited to a single bacterial cell. This issue may be alleviated by the examination of a substantial quantity of individual cells. One instance of such a methodology involves the random confinement of bacteria inside nanoliter droplets, enabling the rapid and efficient analysis of a large quantity of individual

droplets (1). The use of appropriate internal standards is crucial while doing a direct study of clinical samples. Furthermore, any investigation based on growth may be completely unsuccessful if the rate of growth is very small or if the microorganisms stop growing due to the absence of certain growth nutrients, an unfavorable environment, or the buildup of compounds that restrict development.

NAAT may be required for intracellular pathogens. Establishing appropriate growth conditions for tiny set-ups may provide challenges in comparison to typical microbial cultivations. The aforementioned issues may be alleviated by the examination of cell viability, morphology, or motions. The effectiveness and ability of cultivation-based AST may be enhanced by miniaturization, but only if the requirement for manual procedures is reduced. Chemical diluted forms are frequently carried out prior to loading into the chip device, resulting in a comparable degree of set-up complexity to that seen in broth dilution procedures. Miniaturized cultivations are advantageous because they concentrate the cells and eliminate contaminants, which are often present in clinical samples with a low number of pathogens and impurities in the sample matrix. In order to achieve these objectives, the FISH-based Accelerated Pheno technology utilizes an electrokinetic/electrophoretic technology.

Simple inertial microfluidic devices may be used to perform coarse cell sorting for blood specimens. In these systems, bacteria are driven to the outside part of a spiral-formed microcapillary tube by centrifugal (inertial) force, while blood cells remain on the interior (35). Certain techniques mitigate the issue of contaminants by using magnetic and antibody-coupled pellets or nanoparticles to place the target in close proximity to the surface of the biosensor. The process of miniaturization imposes significant requirements on the standardization of conditions; since it necessitates that the samples exhibit comparable growth stages and culture densities. Accurate pipetting may require the use of robots.

There have been significant concerns raised over the capacity of expedited cultures to completely replace conventional growth-based testing. The differentiation between wild type and resistant strains may provide challenges in rapid cultivations (10,36). Increased generation of resistance variables, such as AmpC in enterobacteria or macrolide-resistance in streptococci, is particularly worrisome (37).

6. Factors Stimulating The Expansion Of AST

Rapid AST has the potential to provide significant cost savings by reducing hospital days, disability days, and preserving lives (3). The exorbitant expense of molecular testing is a significant obstacle to the use of new technologies, notwithstanding the potential for significant cost reductions. Molecular testing typically have a price range of $100 to 250 dollars per assay (9). The cost of reagents alone for doing thorough multiplex screening of positive cultures of blood using NAAT in a typical 500-bed regional hospital may exceed $500,000 per year (16). Additionally, the costs of the instruments may be substantial. Estimating the overall expenses associated with testing is a considerable challenge. The pricing structure for laboratory tests and diagnostic tools is determined by the offers provided by distributors.

The expenses associated with testing involve clinical testing as well as the customary cultivations conducted in microbiology laboratories, which involve enrichment and preparation of bacterial isolates, prior to the execution of AST. For instance, Patel et al. (38) conducted an estimation of the overall expenses associated with mass spectrometry-based antimicrobial susceptibility testing (AST). They determined that the combined costs of the MALDI-TOF apparatus, reagents, pharmacist time, and the antimicrobial management program amount to around 79 Euro per patient. However, the cost of the reagent for a single MS-sample may be reduced to around 1 Euro.

According to Wieser et al. (39), the cost of a standard MALDI-TOF MS system, including all associated peripherals, software databases, and maintenance, may reach up to 200,000 Euro annually. This suggests that in order to achieve a satisfactory level of cost efficiency, the usage of the tool must be substantial. Regrettably, this precludes their application in outpatient medical centers. The versatility of MS-instruments allows for their use in many routine diagnostics inside central labs (40). Plates have been tested and used in several clinical labs for direct inoculation into automated identification systems like Vitek, Microscan, and others. These techniques use multiwell liquid cultures as well as exhibit a turnaround period of up to 4 hours for identification and 6-8 hours for assay testing (16).

However, it is worth noting that the costs associated with these equipment are very expensive. The expense associated with a quick AST should not much above the cost threshold now deemed acceptable for regular testing. This range typically falls between thirty to fifty €, including expenses related to sample, culturing, as well as AST. A recent evaluation was conducted to assess the effectiveness and costs of five phenotypic assays designed for the targeted identification of enterobacterial carbapenemases. The application of culture isolates was necessary for all of these assays. According to Baeza et al. (41), the expenses associated with multiplexed PCR-based analysis amounted to 30 € each sample, immunochromatographic techniques cost 15 Euro, a colorimetric assay incurred 5 Euro, and the carbapenem hydrolysis test incurred 1 Euro per sample. In addition to the duration of turnover, the ability of the evaluation system has significance.

Accelerate Pheno is an automated microscopy system that is capable of processing a single sample per unit module, with a maximum of four modules per device. The cost per sample for Accelerate Pheno is 250 Euro. In contrast, the Q-Linea ASTar technology has the capacity to handle up to 50 samples per day. NAAT-based systems have the potential to achieve much greater capabilities. Automated MIC-determination systems, like VITEK-2, have the capability to handle a large number of samples simultaneously. In the context of POCT use, it is deemed adequate to have a throughput capability of several tens of samples each iteration.

In relation to the velocity and necessity of managing clinical polybacterial samples, several promising options for expeditious point-of-care evaluation of antimicrobial resistance include the immunodiagnostic TPX-technology developed by ArcDia Ltd, the growth-based FISH technique employed by Accelelerate Pheno, the Multipath digital imaging technology utilizing nanoparticles for identifying and magnets for capturing, as proposed by First Light Diagnostics

Inc., and the NAAT-based dAST method developed by Talis Inc. However, the Talis system has not yet been introduced to the market. Immunobiosensors are now in their nascent stage of development, although they have potential for future significance in the assessment of non-culturable microorganisms. Lateral Flow systems have the potential to serve as an optimal product format for clinical applications. However, their effectiveness has so far been limited to isolated cultures, namely colonies seen on a plate.

The identification of drug resistance in Gram-negative bacteria and the examination of samples including commensal flora pose challenges for NAAT due to the wide range of resistance mechanisms (10). NAAT may not be able to detect multiple ESBL genes including genes that confer resistance to fluoroquinolones or aminoglycosides. Although NAAT is a potent and essential technique for identifying fastidious, slow-growing, or intracellular pathogens, it is particularly useful for detecting toxin-producing bacteria like E. Both coli O157:H7 and viruses have been studied by Miller et al. (42). However, the growing use of NAAT has sparked apprehension over the destiny of bacterial samples necessary for further investigations (43). Given that NAAT does not need the use of live samples, it may be unfeasible to conduct further epidemiologic investigations or cultivation-based validation AST using the preserved samples. The use of WGS (whole genome sequencing) for fast AST is still in its early stages. The bioinformatics required is complex, and the interpretation of the findings necessitates the use of global open databases.

In the foreseeable future, advancements in chip, microfluidics, and biosensor technologies have the potential to provide cost-effective alternative methods for AST. The resolution of issues pertaining to a low baseline pathogen count and the existence of contaminated sample matrices would need the integration of several advanced technologies. A number of scholarly articles have already provided evidence of the effective utilization of smartphone optics and telecommunication capabilities in the monitoring of microwell or microcapillary cultivations, pH and redox fluctuations, and the transmission of biosensor data read-outs (44,45,46). The evident absence of intellectual property rights (IPR) safeguards for smartphone-based analytical devices, coupled with the need to authenticate these devices as distinct entities, regrettably hinders the commercialization of these inventions.

7. Conclusion

To summarize, the assessment of novel technologies for expeditious diagnostic testing within laboratory settings has significant importance in tackling the worldwide issue of antibiotic misuse and AMR. The existing condition of medical facilities is characterized by a deficiency in a comprehensive and efficient diagnostic system capable of accurately identifying the etiological agent, distinguishing between viral and microbial illnesses, detecting antibiotic resistance, and providing guidance for suitable antimicrobial interventions. The WHO has acknowledged AMR as the foremost global health hazard, underscoring the need for prompt intervention. The study emphasizes the concerning consequences of AMR, as the yearly death toll caused by drug-resistant illnesses has already surpassed 700,000 people and is expected to possibly reach 10 million by 2050. Furthermore, the prevalence of superfluous and unsuitable antibiotic use in

Western countries has escalated to 30%. This underscores the pressing need for enhanced diagnostic instruments in both hospital and non-hospital environments.

This paper investigates the progress made in the field of quick molecular diagnostics, including various approaches such as nucleic acid synthesis, electrochemical methods, microarrays, micro- and nanoparticles, and mass spectrometry applications. Nevertheless, it is important to recognize the restricted range of molecular methodologies that have received approval from regulatory entities like the FDA. Attempts have been undertaken to improve the susceptibility of antimicrobial agents and develop systems for detecting microorganisms. Genotypic methodologies that depend on nucleic acids have the ability to specifically target resistances, however, phenotypic testing is still essential for precise diagnoses. Conventional AST techniques, however efficient, need several days to provide results. Automated turbidity measurement devices and laser-light dispersion advances, which are commercial technology, provide quicker turnaround times for AST findings.

Moreover, the research underscores the need of incorporating these developments in outpatient environments, given that existing diagnostic methodologies are sometimes inaccessible outside the confines of inpatient facilities. The prompt and precise identification of urine and respiratory infections may play a significant role in limiting the spread of AMR bacteria and minimizing unwarranted patient seclusion, leading to financial and resource conservation.

Nevertheless, the use of innovative molecular methods for AST has been hindered by practical constraints and the need for uniformity in antibiotic prescription guidelines across various geographical areas. The research also highlights the need for doctors to engage in organized communication and examination of diagnostic data in order to efficiently harness the advantages offered by technological breakthroughs. In summary, the assessment of novel technologies for expeditious diagnostic testing inside laboratory settings presents encouraging prospects for tackling the worldwide issues of antibiotic misuse and AMR. The promise for revolutionizing infectious disease treatment, reducing antibiotic abuse, and efficiently managing AMR exists via the integration of these innovations into clinical practice, as well as through enhanced communication and analysis.

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