

Review

Advances in Antimicrobial Resistance Monitoring Using Sensors and Biosensors: A Review

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Abstract: The indiscriminate use and mismanagement of antibiotics over the last eight decades have led to one of the main challenges humanity will have to face in the next twenty years in terms of public health and economy, i.e., antimicrobial resistance. One of the key approaches to tackling antimicrobial resistance is clinical, livestock, and environmental surveillance applying methods capable of effectively identifying antimicrobial non-susceptibility as well as genes that promote resistance. Current clinical laboratory practices involve conventional culture-based antibiotic susceptibility testing (AST) methods, taking over 24 h to find out which medication should be prescribed to treat the infection. Although there are techniques that provide rapid resistance detection, it is necessary to have new tools that are easy to operate, are robust, sensitive, specific, and inexpensive. Chemical sensors and biosensors are devices that could have the necessary characteristics for the rapid diagnosis of resistant microorganisms and could provide crucial information on the choice of antibiotic (or other antimicrobial medicines) to be administered. This review provides an overview on novel biosensing strategies for the phenotypic and genotypic determination of antimicrobial resistance and a perspective on the use of these tools in modern health-care and environmental surveillance.

Keywords: antimicrobial resistance; sensor; biosensor; antibiotic susceptibility test; phenotypic technique; genotypic technique



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1. Antimicrobial Resistance

In recent years, antimicrobial resistance (AMR) has received a great deal of attention because of the impact resistant microorganisms have on public health. Consequently, it has been declared as a global public health concern [1–7]. According to the World Health Organization (WHO), antimicrobial resistance occurs when bacteria adapt and grow in the presence of an antimicrobial (AM) agent [8]. It can also be described as a phenomenon when microorganisms (bacteria, viruses, fungi, and parasites) are no longer affected by an AM to which it was previously sensitive, as a result of mutation of the microorganism to evade the effect of the antimicrobial or of the acquisition of the resistance gene [9,10]. It is estimated that by 2050, human deaths related to AMR will amount to 10 million, resulting in an economic cost of about 100 billion dollars [11]. Although, there is compelling economic justification for the development of new generations of antibiotics by 2030 [12], the acquisition rate of biological resistance to new drugs is now a major consideration in preventing their introduction [7,13,14].

The WHO has established a list of antimicrobials (limited to antibiotics) that are important in human medicine and in veterinary practice. The top priority antimicrobials are cephalosporins (3rd, 4th, and 5th generation), glycopeptides, macrolides and ketolides,

polymyxins and quinolones [4,15]. In 2017, the WHO published a list of priority pathogens resistant to antibiotics; on this list, 12 families of bacteria are selected for the research and development of new antibiotics due to the growing need for innovative treatments against multi-resistant bacteria in hospital environments (Figure 1) [16,17].

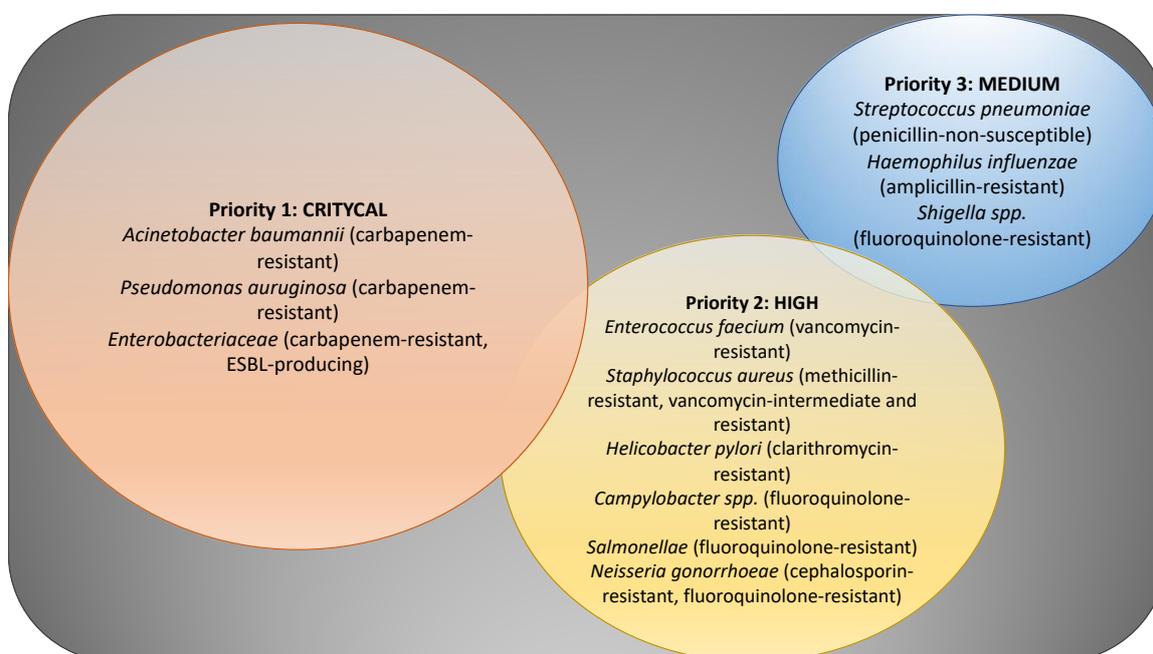


Figure 1. List of priority pathogens for research and development of new antibiotics.

One of the leading causes contributing to the global increase in AMR is the empirical use of broad-spectrum AMs in clinical and veterinary practices [18]. For the early detection of microorganisms, for the prevention of diseases and spread of AMR, it is essential to have well-equipped laboratories specialized in the diagnosis of infections [19]. It is difficult to meet this requirement in developing countries because of the lack of funds, shortage of trained personnel, and lack of the required infrastructures. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, reliable antimicrobial resistance tests require phenotypic evaluation, i.e., experimental procedures to monitor the microbial growth in presence of AM. The routine procedure to determine if a pathogen is resistant is through isolation, including enrichment culture (i.e., urine culture) and plate cultivation, identification and finally antimicrobial susceptibility testing (AST) using different AM loads. The results can be expressed as the lowest concentration of the antibiotic that prevents visible growth, i.e., the minimal inhibitory concentration (MIC) [20]. ASTs based on culturing methods require from 24 to 72 h. This time lapse can be crucial for the patient [21,22]. Another disadvantage of culturing-based techniques is that trained personnel is required [23]. The disk diffusion test, introduced, in the 1960s by Bauer and Kirby to determine the susceptibility to antibiotics in *Staphylococcus strains*, is still one of the most frequently used phenotypic method for AST [24,25]. Broth microdilution assays is another commonly used technique [26,27], based on visually identifiable growth. Automated commercially available AST instruments have helped in reducing the analysis times to less than a day [28]. Examples of automated instruments include the Vitek systems (BioMérieux), the BD Phoenix instruments, the Alfred 60AST system (Alifax), and the MicroScan WalkAway system (Beckman Coulter) [18,25,29–31].

Other candidates for AST are flow cytometry (FC) and isothermal microcalorimetry (IMC). FC is an optical technique used for cell counting and the detection of biomarkers [32]. Since the mechanism of action of antibiotics induces modifications in some morpho-functional and physiological characteristics of the cell, it is possible to determine

the degree of susceptibility with FC [33,34]. The evaluation of AMR by IMC [35] consists of measuring the change in the rate of heat production in the cultural environment in the presence of an AM [36]. One of the advantages of this technique is its non-destructive nature. However, the results can take several hours or even days [37]. Another limitation of IMC is that non-replicating cells in the biofilm or the total biomass are not directly quantified [38].

Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), has been proposed for the identification of pathogens due to its precision. Pathogen identification (ID) is a recommended step of AST. However, one of the disadvantages is that not all hospitals or microbiological laboratories can have this type of instrument, in addition to the fact that cultured clinical samples are always required, which are usually available after 24 h [39,40].

The acquisition of resistance gene can be identified by means of nucleic acid-based methods (genotypic methods) such as the polymerase chain reaction (PCR)-based procedures [41] and other nucleic acid amplification technologies. However, genotypic methods can only find resistances that are searched for, since only selected genes are evaluated. Moreover, DNA extraction is critical so that complex matrices can lead to severe interpretation problems; this means that the potentially found resistance genes are not necessarily from the actual pathogenic organism. PCR-based method can become unspecific if the primers are not well designed or aligned with other regions of the DNA, causing false positives [20,42–44]. Whole genome sequencing (WGS) of the pathogen by using next-generation sequencing (NGS), can provide a complete picture of the pathogen's resistome, such as predicting known resistance phenotypes, but like PCR, this methodology does not express the putative functionality of these genes present in the microorganism. This can be solved with functional metagenomics, but it involves a prolonged time since the genes of interest are inserted into a plasmid and these turn into a host, such as a bacterial strain, continuing with the culturing process and AST, so that the time can be prolonged [9,45]. Another widely used technique for gene resistance evaluation are microarrays technology [46] and fluorescence in situ hybridization (FISH), using synthetic capture probes of different types including peptide nucleic acid (PNA) probes [47–50]. However, for the development of PNA-FISH, biological samples are commonly previously cultured by automated instruments, thus increasing the analysis time.

The use of chemosensor and biosensor technology is an alternative approach which possesses technical simplicity, low cost, short analysis time, easy operation, and hold exceptional promise for providing critical information for real-time data collection and simultaneous measurements of multiple analytes (multiplexing) within a single device. In general terms, chemosensors are devices that measure the signal produced by a chemical reaction whereas biosensors can be defined as “compact analytical devices incorporating a biological or biologically derived sensing element either integrated within or intimately associated with a physicochemical transducer” [18,19,51–58].

Different examples of sensors and biosensors for antimicrobial susceptibility tests and resistance gene detection have been reported in literature [59–64]. Complex real matrices have been analyzed including biological matrices such as saliva, blood, and urine [65,66], environmental matrices such as soil, water (drinking water, rivers, lakes, groundwater, wastewater), plants [67–70], livestock [71], and food [55,67,68].

The aim of this review is to describe sensor- and biosensor-based methods for detecting AMR, limiting the discussion to the system proposed for phenotypic and genotypic AMR detection. Thus, (bio)sensor-based methods for general pathogen identification are not reviewed here.

2. Sensors and Biosensors for AMR Detection

Methods for AMR detection based on sensor and biosensors are here classified as phenotypic and genotypic tools. The phenotypic techniques consist of detecting the expression of the microorganism's resistance mechanisms, i.e., the microorganism survival in presence

of an antimicrobial. Genotypic techniques allow the detection of genes that express some mechanism of the microorganism resistance to the action of the antimicrobial. Many resistance target genes are known and are listed in specialized databases such as The Comprehensive Antibiotic Resistance Database (CARD, <https://card.mcmaster.ca/>, accessed on 17 August 2021), ResFinder (https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/, accessed on 17 August 2021) and Gene (<https://www.ncbi.nlm.nih.gov/gene/>, accessed on 17 August 2021) [9]. Some examples of these genes are reported in Table 1.

Table 1. Main antimicrobial groups, AMR genes, and type of resistance mechanisms [9,72,73].

Antimicrobial Group	Antimicrobial Agent(s)	Some of Resistance Gene(s)	Resistance Mechanism
Aminocoumarins	Novobiocin, Coumermycin, Clorobiocin	gyrB, parE, parY	Target modification
Aminoglycosides	Amikacin, Dibekacin, framycetin, gentamicin, kanamycin, neomycin, netilmicin, plazomicin, sisomicin, spectinomycin, streptomycin, tobramycin	aacA-aphD, aadD, aadE, aadY, ant(4')-Ia, aphA3, armA, rmtA, rmtB, rmtC, str	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Amphenicol	Azidamfenicol, chloramphenicol, florfenicol, thiamphenicol	agmR, catA1, cmlA1, floR, ttgABC	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Ansamycins	Rifampicin, rifamixin	dnaA, rbpA, rpoB	Target modification, antibiotic modification, reduced permeability
Carbapenems	Doripenem, ertapenem, Imipenem	blaOXA-23, blaOXA-58, blaOXA-497, blaVIM-1	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Cephalosporins	Cefacetrile, cefalexin, cefalotin, cefapyrin, cefazolin, cefiximine, cefotaxime, ceftriaxone, cefuroxime	ampC, bla-genes	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Ethambutol	Ethambutol	embB, embC	Expression changes, target modification
Fluoroquinolones	Ciprofloxacin, clinafloxacin, levofloxacin, moxifloxacin, ofloxacin, pazufloxacin, sarafloxacin	gyrA, gyrB, parC, parE	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Fosfomicin	Fosfomicin	fomA, fomB, fosC, fosA, fosB, fosX	Antibiotic modification, target modification
Fusidanes	Fusidic acid	fusB, fusC	Reduced permeability, target protection
Glycopeptides	Corbomycin, ramoplanin, telavancin, teicoplanin, vancomycin	vanA, vanB, vanD, vanR, vanS	Reduced permeability, target modification
Isoniazid	Isoniazid (INH)	ahpC, inhA, katG	Expression changes, target modification
Lincosamides	clindamycin, lincomycin, pirlimycin	ermA, ermB, erm(31), ein, lnu(A), lnu(B), lsa(B), sal(A)	Antibiotic efflux, antibiotic modification, target modification
Lipopeptides	Bacillomycin, caspofungin, daptomycin, mycosubtilin, surfactin, surotomicyn	cdsA, rpoB	Antibiotic modification, target modification
Macrolides	Azithromycin, erythromycin, oleandomycin, josamycin, roxithromycin, spiramycin,	cfr, ermA, ermB, erm(31), ereA, ereB, gimA, mefA, mefE, mel, mgt, ole	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Mupirocin	Mupirocin	mupA, mupB	Target modification

Table 1. Cont.

Antimicrobial Group	Antimicrobial Agent(s)	Some of Resistance Gene(s)	Resistance Mechanism
Nitroimidazoles	Azanidazole, benzinidazole, dimetridazole, megazol, metronidazole, nimorazole, ornidazole, pretomanid, tinidazole	nimA, nimB, nimC, nimD, nimE	Antibiotic modification
Nitrofurans	Furazolidone, nifuroxazide, nifurtimox, nifurtoinol, nitrofurural, nitrofurantoin, nifurzide	nfsA, nfsB	Antibiotic efflux, target modification
Oxazolidinones	Linezolid, posizolid, radezolid, tedizolid	cfr, oprA, poxtA	Antibiotic efflux, target modification
Penicillins	Amoxicillin, ampicillin, benzylpenicillin, cloxacillin, penicillin, phenethicillin	ampC, blaZ, mecA, mecZ	Reduced permeability, antibiotic efflux, antibiotic modification, target modification
Polymixins	Bacitracin, colistin	mcr-genes, mgrB, pmrA, pmrB, pmrE	Antibiotic efflux, target modification, expression changes
Pyrazinamide	Pyrazinamide	clpC1, pncA, rpsA	Target modification
Streptogramins	Quinupristin/dalfopristin, pristinamycin, virginiamycin	cfr, erm-genes, lsa, msrA, vga, vgaB, vatA, vatB, vatC, vatD, vatE	Antibiotic efflux, antibiotic modification, target modification
Sulfonamides	Sulfadiazine, sulfadimethoxine, sulfadimidine, sulfafurazole, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfapyridine	sul1, sul2, sul3, sul4	Reduced permeability, expression changes, target modification
Tetracyclines	Chlortetracycline, doxycycline, omadacycline, oxytetracycline, tetracycline	tetA, tetB, tetC, tetM, tetO, tetQ, tetX, tet30, tet31, tet32, tet36	Reduced permeability, antibiotic efflux, target protection, target modification
Trimethoprim	Trimethoprim	dfrA, dfrD, dfrG, dfrK	Target modification

2.1. Sensors and Biosensors for Phenotypic AMR Detection

The previous decade has seen a notable increase in the development of chemical sensors and biosensors for phenotypic AMR detection [18,74–77]. Herein, sensors and biosensors for AMR testing have been classified according to the transducer used, and thus are discussed as magnetic, mass, mechanical, thermal, optical, and electrochemical. Mechanical sensors and biosensors are devices sensitive to physical changes in mechanical properties and capable to convert interactions and processes occurring at their surface into measurable mechanical properties [76]. Optical sensors are based on the measurement of reflectance, chemiluminescence, absorbance, fluorescence, Raman scattering, and surface plasmon resonance (SPR) [78–81]. SPR is one of the most widely used optical label-free platforms [24,82]. Electrochemical (bio)sensors can measure potentiometric, amperometric, voltametric, or impedimetric signals [51,75,83–86].

2.1.1. AST Magnetic, Mass, and Mechanical (Bio)Sensors

The magnetic properties of some materials have been explored for AMR monitoring. Asynchronous magnetic bead rotation (AMBR) is one of these. In this technique, the rotational rate of magnetic beads in a rotating magnetic field is monitored. The measurement is based on the changes in the rotation frequency given by the physical properties of the bead itself (shape and volume) and its environment (viscosity and bacterial load) [87]. The number of bacterial cells present in a sample affects the rotation frequency. In presence of an antibiotic, the MIC of the antibiotic can be estimated [88]. Kinnunen et al. measured the bacterial growth of uropathogenic *E. coli* isolate and its response to the presence of

streptomycin and gentamicin by coating magnetic beads with specific antibodies for the bacteria. Bacterial growth alters the drag of the rotating magnetic bead cluster due to the change in viscosity (Figure 2A) [89]. With this device, the MICs of streptomycin (8 µg/mL) and gentamicin (2 µg/mL) were obtained in 2 h.

Other devices that also use beads are those based on the measurement of the Brownian motion. Wang et al. developed a self-powered sensor based on vancomycin-modified microbeads and the use of their Brownian motion for rapid AST and in situ monitoring of microorganisms [90]. The proposed approach is based on the fact that vancomycin is a β-lactam antibiotic that possesses a similar structure to the enzyme dd-transpeptidase that catalyzes the synthesis of bacterial cell walls. Cell walls of almost all bacteria contain d-Ala-d-Ala residues. Gram-negative bacteria are covered with an extra layer of outer membrane, however d-Ala-d-Ala ligases still are exposed to the outside environment through some defects [91]. Thus, the vancomycin-modified microbeads can selectively capture bacteria instead of normal cells or proteins and inhibit their wall synthesis. When the functionalized microbeads capture the bacteria, giving rise to a change in diffusivity, the Brownian motion is weaker; the images of the analysis were then obtained by means a fluorescent microscope. In this study, *E. coli* and *S. aureus* strains were used, and gentamicin was added for AST. It was determined that concentrations below 2 µg/mL were insufficient to inhibit the bacteria; the determined MIC was 4 µg/mL with a detection limit of 10⁵ CFU/mL in an analysis time of 2 h and a volume of 5 µL.

Quartz crystal microbalance (QCM) allows piezoelectric/acoustic dynamic measurements. This technique measures the resonant frequency change from mass changes on the transducer surface. The surface of the transducer is a piezoelectric material; quartz is commonly used for its low cost and stability against thermal, chemical, and mechanical stresses [92]. The quartz crystal is coated with a gold electrode; antibodies, DNA or RNA capture probes, enzymes or nanostructures that are specific and highly sensitive to the substance to be detected can be immobilized on the gold surface [93,94]. Reyes et al. reported a rapid and highly sensitive biosensor for monitoring the effects of antibiotics on resistant or susceptible strains of *E. coli* and *S. cerevisiae* [95]. A magnesium zinc oxide (MZO) nanostructure-modified quartz crystal microbalance (MZO nano-QCM) was used (Figure 2B). The biosensor was applied to measure the sensitivity of the strains to ampicillin and tetracycline for *E. coli*, and amphotericin-B and miconazole for *S. cerevisiae* through the device's time-dependent frequency shift and motional resistance [95]. An innovative alternative for the determination of AST was proposed by Guliy et al. [96]. The principle of operation of the sensor is based on the fact that the attenuation of an acoustic wave propagating in a piezoelectric plate and its velocity depend on the electrical boundary conditions on the surface of the plate [97]. In the proposed approach the lower part of a lithium niobate plate was excited with interdigital transducers (IDT), the signal generated was determined in the form of an acoustic wave with horizontal polarization; the suspension of *E. coli* cells, placed on top of the lithium niobate plate, led to the appearance of pronounced resonance peaks. Subsequently, the bacterial suspension was exposed to ampicillin and measurements of the depth and frequency of the resonant peaks were made; at 2 µg/mL of antibiotic, a change in the depth and frequency of the resonance peaks can be observed because ampicillin modifies the properties of the cytoplasmic membrane, favoring the release of species to the suspension by modifying its conductivity; this increase in conductivity decreases the depth and frequency of the resonant peaks of the sensor. This device has the advantage of short analysis times (10–15 min). However, it is affected by temperature changes, causing fluctuations in the resonance frequency.

Mechanical sensors have been proposed for AST [76,98]. The forces produced by the interaction of the target species with the receptors immobilized on the cantilever determine a change in the cantilever resonance frequency that behaves as a harmonic oscillator. For the determination of bacteria and their susceptibility or resistance, the cells adhere to the cantilever, and according to the added mass and position, the change in resonance frequency is monitored [74,99,100]. Currently, the atomic force microscope

(AFM) is one of the main nanomechanical techniques used in AST due to its ability to detect displacements in the range of 0.1 \AA and a resolution of microseconds [98,101]. Using an AFM system, Stupar et al. developed a methodology capable of detecting nanomotion of *E. coli* bacteria and observing their behavior when exposed to ciprofloxacin, ampicillin, and ceftriaxone [102]. The bacteria themselves induce a fluctuation in the sensor; when exposing the bacteria to antibiotics, a change in the fluctuations is rapidly recorded (Figure 2C).

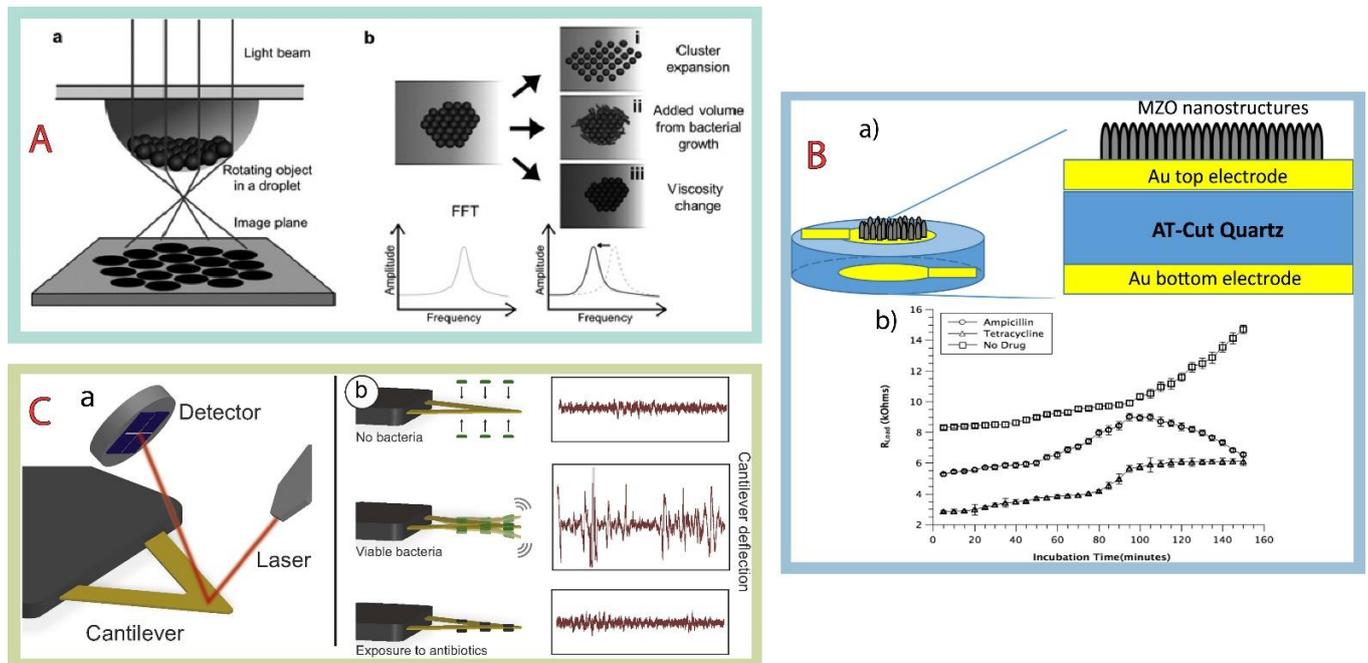


Figure 2. Examples of different magnetic, mass and mechanical (bio)sensors: (A) Schematic of an AMBR biosensor. (a) The droplet lensing effect was used to amplify the rotation signal; the rotation signal can be observed using a photodetector and visualizing a periodic signal that corresponds to the rotational period of the cluster. (b) The rotational period of the cluster is different when (i) the cluster expands, (ii) the bacteria attach to the cluster, or (iii) the viscosity of the surrounding fluid changes. Reprinted from reference [89] with permission from John Wiley and Sons. (B) (a) Scheme of the MZO nano-QCM biosensor; (b) the motional resistance (R_{load}) is calculated from the sensors admittance spectrum amplitude modulation for three cases, i.e., ampicillin, tetracycline, and no drug treatment. Reprinted from reference [95] with permission from Elsevier. (C) Representation of nanomechanical sensor based on cantilever. (a) Schematics of nanomotion detector setup; laser beam is focused on the sensor surface, and the movements of the cantilever are monitored by evaluating the reflection. (b) Representation of a nanomotion susceptibility test. The fluctuations are driven only by thermal motion and are low, when bacteria are not attached to the sensor. After attachment of bacteria, fluctuations are linked to their metabolic activity and are high. Finally, after exposure to the antibiotic, bacteria are nonviable, and fluctuations return to low levels. Reprinted from reference [102] with permission from Elsevier.

Table 2 summarizes some of the most recent works for the determination of susceptibility through magnetic, mass, and mechanical sensors. Mainly, these sensors were tested in bacterial culture. Thus, in Table 2, detection times after the isolation and identification of the strains are detailed, as well as the MICs of the antibiotics used for susceptibility or resistance measurement and the detection limits.

Table 2. Different magnetic, mass, and mechanical sensor for AST.

Technic/Signal/Type	Target	Antibiotic	MIC ($\mu\text{g/L}$)	Limit of Detection	Time	Reference
Asynchronous magnetic bead rotation	<i>E. coli</i>	Gentamicin	1	Single bacterium binding events	15 min	[87]
Atomic force microscope cantilevers	<i>E. coli</i>	Ampicillin	12.5–50	NM, 1×10^5 CFU/mL **	45 min	[100]
Asynchronous magnetic bead rotation	<i>E. coli</i>	Ampicillin	8	Single bacterium binding events	1.5 h	[103]
Asynchronous magnetic bead rotation	<i>E. coli</i>	Gentamicin	1	50 cells per drop	100 min	[104]
Asynchronous magnetic bead rotation	<i>E. coli</i>	Gentamicin	2	Single bacterium binding events	91 min	[105]
Brownian motion	<i>P. aeruginosa</i>	Gentamicin	2	One bacterium	2 h	[106]
Multi-channel series piezoelectric quartz crystal	<i>E. coli</i>	Ampicillin	32	5×10^5 CFU/mL **	5–8 h	[107]
Spectral amplitude modulation MZO-QCM	<i>S. epidermidis</i>	Ciprofloxacin	0.5	1×10^5 CFU/mL	1.5 h	[108]
		Oxacillin	1			
		Ciprofloxacin	1			
Orthogonal quartz crystal microbalance	<i>E. coli</i>	Ciprofloxacin	12.5	5×10^8 CFU/mL**	1 h	[109]
		Ceftriaxone	15			
		Tetracycline	150			
Indirect series piezoelectric	<i>M. tuberculosis</i>	Isoniazid	0.1	1×10^3 CFU/mL **	>1 day	[110]
		Rifampin	1.0			
		Ethambutol	2.5			
		Streptomycin	2.0			
		Capreomycin	10			
		p-Aminosalicylic acid	2.0			
		Ethionamide	5.0			
Rifabutin	0.5					
QCM under an external magnetic field	<i>D. desulfotomaculum</i>	Vancomycin	NR	1.8×10^4 CFU/mL **	30 min	[111]
Cantilever NMS under an external magnetic field	<i>M. bovis</i> , <i>M. abscessus</i>	Amikacin	1.7	100 bacterial cells	30 min	[112]
		Rifampin	0.15			
		Isoniazid	0.17			
Atomic force microscope cantilevers	<i>E. coli</i> , <i>S. aureus</i>	Ampicillin	2.0	4.6 ± 0.5 bacteria/100 μm^2	30–40 min	[113]
		Kanamycin	70.0			
biomaterial microcantilever with an embedded microfluidic channel	<i>E. coli</i>	Ampicillin Kanamycin	NM	1×10^5 CFU/mL **	30 min	[114]
Atomic force microscope cantilevers	<i>B. Pertussis</i>	Erythromycin	0.06	NM	20–40 min	[115]
		Clarithromycin	0.12			

** Initial concentration; CFU, colony-forming unit; MZO, magnesium zinc oxide; NM, not measured; NMS, nanomotion sensor; NR, not reported; QCM, quartz crystal microbalance.

2.1.2. AST Optical (Bio)Sensors

Optical (bio)sensors include a wide variety of transducing methods such as fluorescence, chemi- and bioluminescence, colorimetry, surface plasmon resonance (SPR), evanescent optical-planar waveguide, reflectometric interference spectroscopy, etc., [19,116,117].

Colorimetric sensors offer a number of advantages including: simple procedure, low cost, and the possibility of observing color changes with the naked eye or with simple instrumentation in the case of quantitative analysis [70,118]. The use of chemical species that changes color with respect to bacterial growth, such as Resazurin [19,119], or the use of enzymes, such as Horseradish Peroxidase (HRP), that modifies substrates according to the number of microbial cells, can help to measure susceptibility in real-time [70].

In the approach of Sun et al. [120], *p*-benzoquinone was used to monitor the bacterial concentration and specifically distinguish *E. coli* from four other common bacteria, namely, *Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus mutans* (*S. mutans*), and *Salmonella pullorum* (*S. pullorum*). A visible color change, evaluated without any complex instruments but with a simple smartphone, is used for the determination of the concentration of the bacteria.

Many (bio)sensors are based on fluorescent labels including nanomaterials [70,121,122]. Nazemi et al. developed a rapid approach to evaluate antibiotic susceptibility of *E. coli* based on photoluminescence emission of GaAs/AlGaAs quantum well (QW) biochips [123]. Bacterial cells were captured on the surface of the QW, delaying the photoluminescence maximum. The biochips were functionalized with polyclonal antibodies for *E. Coli*. After 4 h of incubation (LB broth, penicillin, or ciprofloxacin), the position of photoluminescence maximum was measured. The photoluminescence maximum depends on the photocorrosion process due to the immobilized bacteria on the surface of the GaAs/AlGaAs biochips (Figure 3A). When bacterial cells are affected by antibiotic exposure, peak photoluminescence occurs faster than in presence of bacteria in LB.

Tang et al. [124] studied the change in pH due to the rapid accumulation of metabolic products during cell growth. A microfluidic pH sensor manufactured by integrating pH-sensitive chitosan hydrogel with poly(dimethylsiloxane) (PDMS) microfluidic channels was used in their study. To observe the change in pH, Fourier transform reflectometric interference spectroscopy (FT-RIFS) was used. The suspension of the bacterial cells (*E. coli*, 1×10^6 CFU/mL) with different antibiotics (azithromycin, amikacin, ciprofloxacin, and tetracycline) was injected into the device measuring the reflectance spectra. The increase in EOT was monitored in real-time by determining the possible inhibition of bacterial growth by different antibiotics (Figure 3B). The technique was adequate to monitor microbial growth and MIC of antibiotics in real-time (azithromycin 1 μ g/mL, amikacin 16 μ g/mL, ciprofloxacin 0.25 μ g/mL, and tetracycline 0.5 μ g/mL) in 60 min.

Rapid analysis of antibiotics susceptibility (ceftazidime, cefotaxime, ampicillin, amoxicillin, levofloxacin, and doxycycline) was demonstrated in *P. aeruginosa* and *E. coli* bacteria by Nag et al. [125] by using localized SPR phenomenon and gold nanoparticles. The analysis consisted of capturing the bacteria (10^5 CFU/mL) on a gold nanoparticle-coated optical fiber sensor directly from urine (Figure 3C). Subsequently, the bacteria were exposed and induced to cell lysis with different antibiotics for 2 h, observing the change in absorbance with time and degree of susceptibility or resistance of the bacteria to the different antibiotics (Figure 3C). The sensor was also tested in tap water samples [125].

Raman spectroscopy (RS), is commonly used to measure molecular vibrational, rotational, and other low-frequency modes providing characteristic information about the effects of AMs on microbial cells [126]. Intensity and wavenumber of anelastic-scattered light of RS can be used to analyze living cells without labeling or fixation procedures. Surface-enhanced Raman scattering (SERS) can be used to amplify the response [28,74,127,128]. Bi et al. developed an AST procedure based on SERS-active Au@Ag core-shell nanorods (Au@AgNR) [129]. The manufacture of the SERS nanotag system consisted of the functionalization of the Au@AgNR with streptavidin (SA) and their interaction with biotinylated antibodies specific for *E. coli* to form the complete SERS nanotags (Au@AgNR@SA@Ab) (Figure 3D). Bacterial cells were trapped by the nanotags (Figure 3D) and measured at different CFU concentrations to check the biosensor sensitivity. Finally, the bacterial cells were exposed to ampicillin to observe the susceptibility of the bacteria. The technique showed a detection limit of 10^2 CFU/mL, an estimated analysis time lower than 4 h and the possibility to analyze complex biological matrices such as blood. Some of the most recent publications related to optical (bio)sensors are summarized in Table 3.

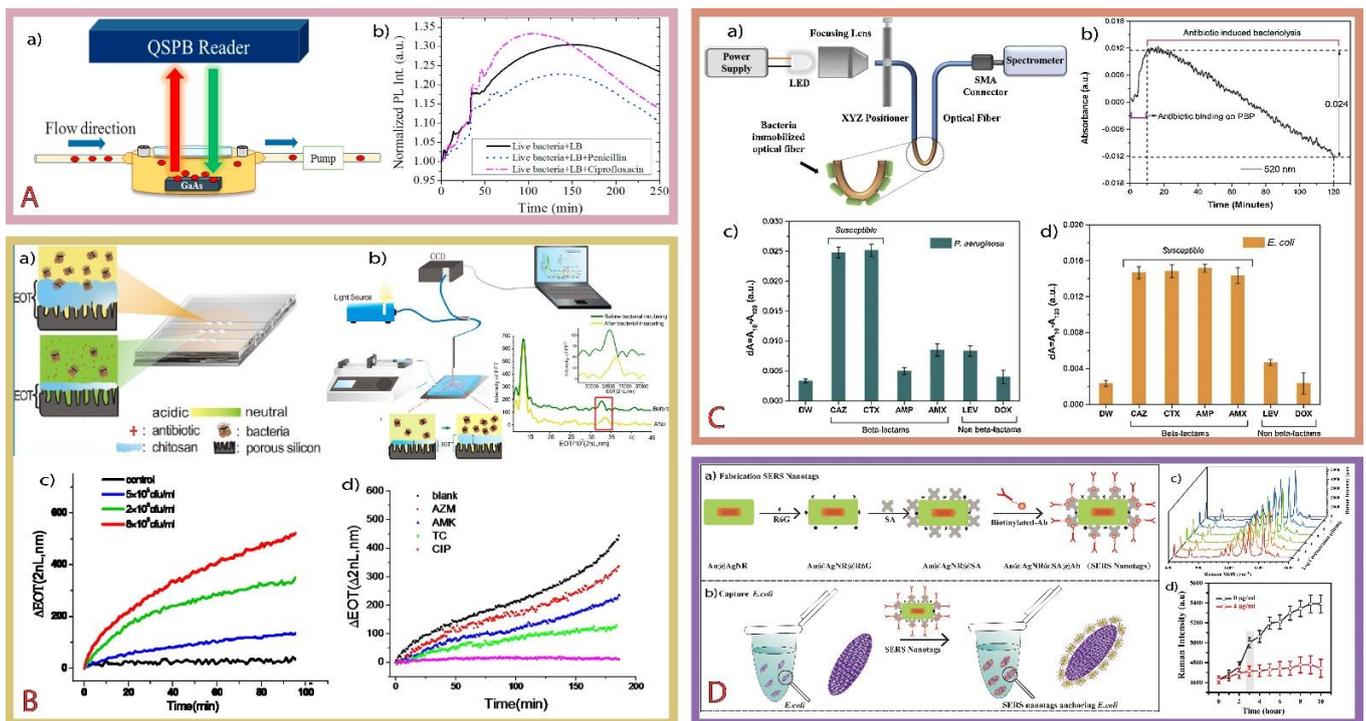


Figure 3. Examples of different optical (bio)sensors for AST. (A) (a) Schematic illustration of the experimental setup; (b) normalized photoluminescence intensity measured for biochips in *E. coli* exposed to penicillin and ciprofloxacin. Reprinted from reference [119] with permission from Elsevier. (B) (a) Mechanism for monitoring bacterial metabolism and growth; (b) schematic instrumental setup for the sample detection; (c) time traces of EOT changes during bacterial incubation in the microfluidic channel; (d) EOT changes measured by the sensor loaded with bacteria spiked with different concentrations of antibiotics. Adapted with permission from (Tang et al., 2013). Copyright (2013) American Chemical Society. (C) (a) Optical detection assembly; (b) time-varying response of *P. aeruginosa* immobilized optical fibers to 10 µg/mL ceftazidime; (c) response of *P. aeruginosa*; and (d) *E. coli* immobilized optical fiber to 10 µg/mL of antibiotics ceftazidime (CAZ), cefotaxime (CTX), ampicillin (AMP), amoxicillin (AMX), levofloxacin (LEV), and doxycycline (DOX). DW is the change in absorbance in distilled water only. Reprinted from reference [121] with permission from Elsevier. (D) (a) Scheme of the functionalization process of Au@AgNR as SERS nanotag; (b) attachment of SERS nanotag to *E. coli*; (c) SERS spectra of *E. coli* complex at a different concentration ranging from 107 to 102 CFU/mL; (d) growth curve of *E. coli* from SERS plot for ampicillin (4 µg/mL) exposure and unexposed. Reprinted from reference [125] with permission from Elsevier.

Table 3. Optical (bio)sensors for determination of susceptibility to antimicrobials.

Technique	Recognition Probe	Target	Antibiotic	MIC (µg/L)	Limit of Detection	Time	Reference
Colorimetric	Tetrazolium salts-8	<i>E. coli</i>	Ampicillin	128	10 CFU/mL	2 h	[122]
Fluorescence	TDN-aptamer/SYTO 9 Green	<i>E. coli</i>	Kanamycin	4.0	10 CFU/mL	5 h	[130]
			Streptomycin	8.0			
			Oxofloxacin	0.5			
			Norfloxacin	1.0			
			Chloramphenicol	2.0			
SPR	2PAC—Au nanosphere/block copolymer templates (PS- <i>b</i> -PMMA)	<i>E. coli</i> , <i>P. aeruginosa</i>	Carbencillin Gentamicin Rifampicin	100 1 Resistant	NR	30 min	[131]
laser-patterned paper-based	Chromogenic agar CHROMagar/photopolymer DeSolute®	<i>E. coli</i>	Amoxicillin	30	2.5 × 10 ⁹ CFU/mL	18 h	[132]

Table 3. Cont.

Technique	Recognition Probe	Target	Antibiotic	MIC (µg/L)	Limit of Detection	Time	Reference
SPR	Poly-L-lysine/glass slide coated with gold sensor chip	MRSAMSSA	Cefoxitin	32 to >128 1 to 4	5×10^5 CFU/mL *	3 h	[133]
Colorimetric	Endogenous H ₂ S/AgNRs	<i>E. coli</i>	Ampicillin	100 (MBC)	10^2 cell/mL *	4–6 h	[134]
Fluorescence	Resazurin	<i>E. coli</i>	Gentamicin	4	Single cell	1 h	[135]
Fluorescence imaging	anti- <i>E. coli</i> antibody/streptavidin-coated polystyrene microsphere	<i>E. coli</i>	Ceftazidime Levofloxacin	4 32	Single cell	30 min	[136]
SERS	Gold nanoparticles	<i>L. lactis</i>	Ampicillin Ciprofloxacin	NR	NR	1.5 h	[137]
Fluorescence	PDMS/TLFM	<i>E. coli</i>	Ampicillin Cefalexin Chloramphenicol Tetracycline	8 12 8 2	Single cell	2–4 h	[138]
SPR	Poly-L-lysine/Au thin film	<i>E. coli</i> <i>S. epidermidis</i>	Ampicillin Tetracycline	3 10	NR	2 h	[139]
SERS	Bacteria-aptamer/AgNPs	<i>E. coli</i> <i>S. aureus</i>	Tigecycline Vancomycin	0.02 0.2	5×10^3 CFU/mL *	2 h	[140]
Raman tweezers	fused-silica microfluidic chip	<i>S. aureus</i>	Oxacillin	2000	10^{12} cells/L	4 h	[141]

* Initial concentration; 2PAC, two-dimensional physically activated chemical assembly method; AgNPs, silver nanoparticles; AgNRs, silver nanorods; MBC, minimal bactericidal concentration; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; NR, Not reported; PDMS, polydimethylsiloxane; PS-*b*-PMMA, poly(styrene-*block*-methyl methacrylate); SERS, surface-enhanced Raman spectroscopy; SPR, surface plasmon resonance; TDN, tetrahedral DNA nanostructure; TLFM, time-lapse fluorescence microscopy.

2.1.3. AST Electrochemical (Bio)Sensors

Electrochemical sensors have also been proposed in AST tests [43,75,142].

Bacteria can be electroactive and carry out a great variety of redox reactions. Thus, electrochemical sensors can determine the electrical response of microbial cells with great sensitivity, in some cases, eliminating the prolonged isolation and culturing times and allowing the measurement directly in biological and environmental matrices [75,143]. Depending on the interaction of the microbial cell with the recognition element, the response can be measured indirectly or directly [144]. In direct detection, the binding of the cell itself with an antibody or a bacteriophage immobilized on the electrode, produces a reduction or increase in the transfer of electrons due to the presence of membrane enzymes [145]. In the indirect detection, the cell is stimulated to secrete some electroactive species that can be measured [144]. Screen-printed electrodes (SPE) have a significant advantage over traditional electrochemical cell methods since the three electrodes (working, counter, and reference electrode) are integrated into a single chip, facilitating the analysis and reducing manufacturing costs and time [146–148]. Hannah et al. implemented an SPE to monitor bacterial growth and select the correct antibiotic to administer to treat infections caused by *E. coli* at a low-cost diagnosis (Figure 4A) [149]. A gold electrode was modified with an antibiotic-seeded hydrogel to measure the resistance to current flow presented by *E. coli* strains by electrochemical impedance spectroscopy (EIS). The bacteria were exposed to streptomycin (MIC of 4 µg/mL) for 2.5 h showing a sensitivity of $\geq 10^5$ CFU/mL.

Furthermore, electroanalytical techniques can be used for pathogen identification significantly reducing the susceptibility determination times [66,150,151]. Altobelli et al. [150] developed an assay for phenotypic antimicrobial susceptibility testing and rapid uropathogen identification. The quantitative determination of bacterial 16S rRNA is based on the use of an electrochemical array functionalized with a panel of complementary DNA capture probes. Pathogen ID was determined using pathogen-specific probes, whereas phenotypic

AST with ciprofloxacin MIC was determined using an Enterobacteriaceae probe to measure 16S rRNA levels as a function of bacterial growth. Results are obtained in very short time ($\cong 6$ h) in comparison to 2–3 days required for the standard diagnosis.

Mach et al. developed an electrochemical, point of care device for determining the antibiotic susceptibility of pathogens of urinary tract directly in urine samples [152]. In this case, each of the working electrodes of a sensor array was modified with a capture probe specific for the bacterial 16S rRNAs of clinically relevant bacterial urinary pathogens. Detection of the target-probe hybrids was achieved through binding of HRP-conjugated anti-fluorescein antibody to the detector probe. Uropathogenic *E. coli* was cultured, and samples taken at time intervals of 20 min for the analysis by standard quantitative plating as well as the biosensor assay. Proportional increases in signal strength were observed over a 4-log unit range. A similar correlation between increased cell number and biosensor signal was observed with other common uropathogens including *E. faecalis* and *P. aeruginosa*. The specific probes for *E. coli* were immobilized on the microfabricated sensor with a thin, optical-grade layer of gold electrodes deposited on plastic. Subsequently, the strains were cultured for 2.5 h in different antibiotics (ampicillin, ciprofloxacin, trimethoprim/sulfamethoxazole gentamicin, ceftriaxone, and cefepime). After this time, cell lysis was performed, and amperometric current versus time was measured using a multichannel potentiostat to observe the susceptibility or resistance of the microbiological species [152]. In the analysis of samples directly from urine, it was determined that the identified *E. coli* bacteria were resistant to ampicillin, ciprofloxacin, trimethoprim/sulfamethoxazole, and gentamicin and susceptible to ceftriaxone and cefepime. A similar approach was reported in [153]. Genefluidics Inc. (Duarte, CA, USA) commercializes a system based on this approach, as also described in the Section 3.

Complementary to oligonucleotide-based capture probes, antibodies are an excellent tool for detecting whole cells due to their high specificity and potential for ID [154]. Recently, Shi et al. implemented a biosensor based on the capture antibody-bacteria-detection antibody sandwich immune complex to demonstrate a culture-free approach and to achieve a rapid diagnosis of bloodstream infections (Figure 4B) [155]. Here, redox electroactive enzymes attached to antibodies are used as sensing elements; the device consists of a three-electrode cell modified with insulated gating electrodes (GE) for applying a gating voltage (VG) between the gating electrode and the working electrode. VG modifies the interfacial charge distribution by inducing an electric field at the solution-enzyme-electrode interface to reduce the tunnel barrier for electrons. A first antibody (specific for *E. coli*) is immobilized on the surface of the working electrode; the sample is incubated with the biosensor; finally, another antibody marked with HRP is added. Cyclic voltammetry (CV) was used to measure the peak reduction of HRP. The *E. coli* bacteria were incubated in the presence of ampicillin to measure whether the strains were susceptible or resistant to the antibiotic. The system allows the determination of bacterial cells in the order of 8 CFU/mL in 1–2 h.

Aptamers have also been proposed for bacterial cell determination [156–159]. To measure, in real-time, the growth and susceptibility of *E. coli* and *S. aureus* to the antibiotics gentamicin, ampicillin, and tetracycline, Jo et al. reported an aptamer-functionalized capacitance sensor array [160]. The specific DNA aptamers for each of the different strains were immobilized on the surface of the Au-working electrode. Bacteria can act as capacitors connected between electrodes in parallel, providing valuable information on bacterial activity. When bacteria are treated with antibiotics at inhibitory concentrations, the capacitance decreases. When the bacteria is resistant to the antibiotic, the capacitance increases in the same way as if the strains were not exposed to the antibiotic (Figure 4C); the analysis can be performed in 1 h with a sensitivity of 10 CFU/mL [160].

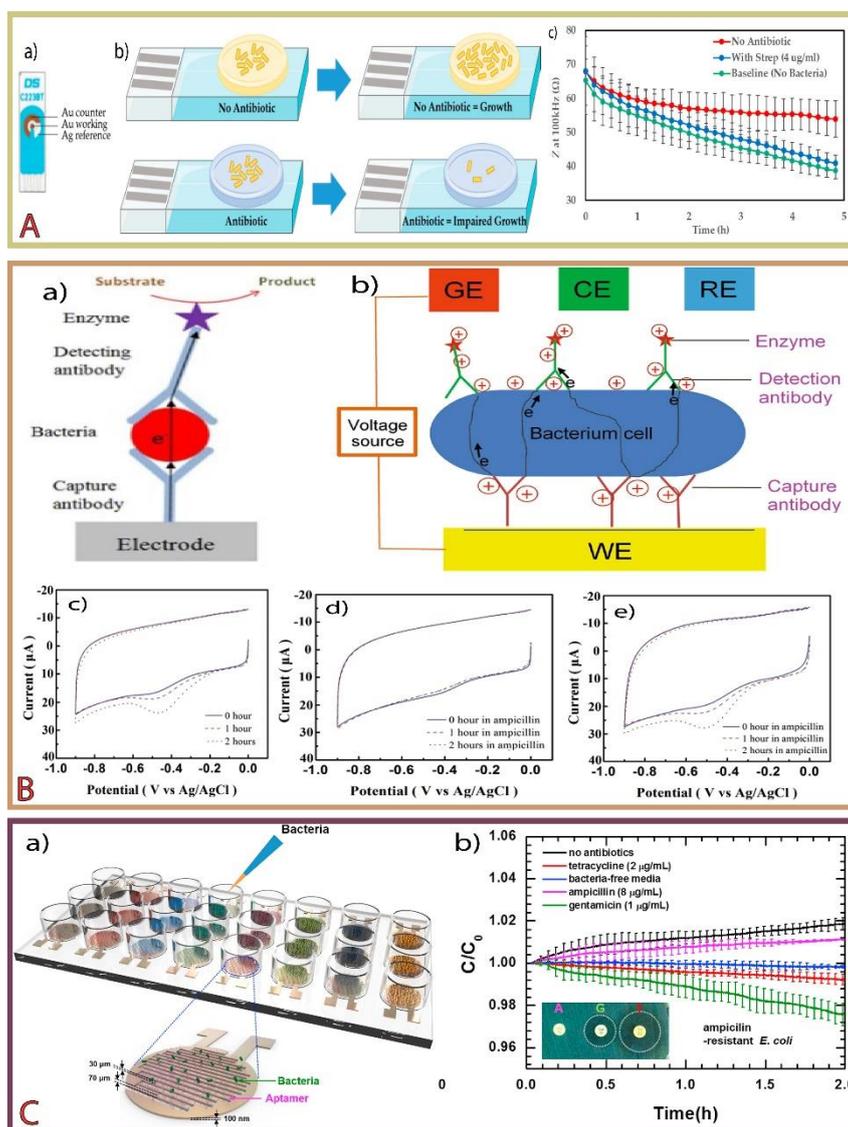


Figure 4. Examples of AST electrochemical sensors. **(A)** (a) Schematic of Au SPE; (b) overview of bacterial growth on the biosensor containing no antibiotic (above) and antibiotic (below) over time; (c) bacterial growth curves of *Escherichia coli* on gels seeded with and without streptomycin and baseline curve (no bacteria). This article [149] is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>, accessed on 17 August 2021). **(B)** (a) The sandwich immune complex formed on an electrode; (b) a schematic description of the FEED-based detection platform; (c) CV of wild-type *E. coli* without ampicillin; (d) CV of wild-type *E. coli* growth in ampicillin; (e) CV of ampicillin-resistant *E. coli* growth in ampicillin. This article [155] is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>, accessed on 17 August 2021). **(C)** (a) Schematic of aptamer-functionalized capacitance sensor array; (b) real-time capacitance measured for ampicillin-resistant *E. coli* treated with tetracycline, gentamicin, ampicillin, or medium. Reprinted from reference [160] with permission from Elsevier.

Graphene field effect transistors (G-FETs) have been gaining attention due to their high sensitivity in the detection of biomarkers and DNA, scalability, biocompatibility, and ease of incorporation of many kind of substrates [161,162]. However, the use of G-FET sensors for bacterial detection counts only a small number of papers describing the detection of a lab strain of *E. coli*, but no reports on the sensing of clinically relevant pathogenic bacteria, nor

on antibiotic resistant strains [163,164]. A rapid, selective, and single cell electrical system for the detection of antibiotic-resistant bacteria based on G-FET was recently developed by Kumar et al. [165]. In their approach pyrene-conjugated selected peptides immobilized on G-FETs were used for detecting pathogenic *S. aureus*. A similar device was also proposed to discriminate between antibiotic resistant and sensitive strains of *Acinetobacter baumannii*, suggesting that these devices can also be used for detecting antibiotic resistant pathogens. In order to enhance the bacteria attachment, electric-field-assisted binding was used, i.e., bacteria were pushed into the graphene transducer by using electrical pulses. This strategy allowed reducing the detection limit to 10^4 cells/mL and the detection time to below 5 min. Table 4 shows some electrochemical sensors developed in recent years and different recognition elements used to determine antimicrobial susceptibility.

Table 4. Electrochemical (bio)sensor for determination of susceptibility to antimicrobials.

Electrode—Recognizing Element	Respond	Target	Antibiotic	MIC ($\mu\text{g/L}$)	Limit of Detection	Time	Reference
Au SPE—antibody alkaline phosphatase	DPV	<i>S. aureus</i>	MRSA strain	Nm	845 CFU/mL	4.5 h	[143]
SPE—Thiolated oligonucleotide capture probes	Amperometric	<i>E. coli</i>	Ciprofloxacin	2	10^3 CFU/mL	5 h	[150]
G-FET—peptide probes	Dirac voltage	<i>S. aureus</i> , <i>A. baumannii</i>	colistin resistant strain	NM	10^4 cells/mL	5 min	[165]
SPCE/MWCNTs/AuNPs—reduction of resazurin	DPV	<i>S. gallinarum</i>	Ofloxacin Penicillin	32 16	10^2 CFU/mL	1 h	[166]
Au—aptamers	Capacitance	<i>E. coli</i> *, <i>A. baumannii</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , <i>E. faecalis</i>	Amikacin Ampicillin Aztreonam Cefepime Cefotaxime Ceftazidime Gentamicin	≤ 2 ≥ 32 ≤ 1 ≤ 1 ≤ 4 ≤ 1 ≤ 1	10^5 CFU/mL **	6 h	[167]
Au SPE—agarose-based hydrogel deposit	EIS	<i>S. aureus</i>	Amoxicillin Oxacillin	8 in both	10^7 CFU/mL (50,000 CFUs) **	45 min	[168]
Pt deposited over a glass substrate—reduction of resazurin	DPV	<i>E. coli</i> , <i>K. pneumoniae</i>	Ampicillin Kanamycin Tetracycline	NM	10^4 cells/mL	4 h	[169]
Two working electrodes (Au and Pt)—POA detection	CV	<i>M. tuberculosis</i>	Pyrazinamide	NM	40 μM of POA	NR	[170]
Array of interdigital electrodes—FD of the impedance of living and dead microorganisms	Impedance	<i>E. coli</i>	Ceftazidime Ceftriaxone Benzylpenicillin	NR	NR	2 h	[171]
Silicon nanowire FETs	Current caused by varying pH values	<i>E. coli</i>	Kanamycin Cefotaxime Ofloxacin	1–4 0.1–6 5	Single cells	6 h	[172]
3-APBA modified electrode bind with cis-diol groups on the cell wall	Capacitance	<i>E. coli</i> , <i>S. typhi</i> , <i>P. aeruginosa</i> , <i>S. epidermidis</i> , <i>S. aureus</i> , <i>B. subtilis</i>	Ceftriaxone Q. infectoria Ampicillin Vancomycin Rhodomycrone	0.03 20 0.5 1.25 0.5	10^8 CFU/mL	2.5 h	[173]
SPE plastic-based microchips—antibodies	Impedance	<i>E. coli</i> , <i>S. aureus</i>	Erythromycin	0.1	10^3 CFU/mL **	1.5 h	[174]
Interdigital electrodes—antibodies	Impedance	<i>S. aureus</i>	Flucloxacillin	100	10^4 cells/mL **	2 h	[175]

* Antibiotic and MIC correspond to *E. coli*; ** Initial concentration; 3-APBA, 3-aminophenylboronic acid; AuNPs—gold nanoparticles; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; FD, frequency dependence; G-FET, graphene field effect transistors; MRSA, methicillin-resistant *Staphylococcus aureus*; MWCNTs, multiwalled carbon nanotubes; Not measured; Not reported; POA, pyrazinoic acid; SPCE, screen-printed carbon electrodes; SPE, screen-printed electrodes.

2.2. Sensors and Biosensors for Genotypic Antimicrobial Resistance Detection

Microorganisms have developed different biological mechanisms to adapt and survive AM attack; these mechanisms are conserved and transmitted to other microorganisms through resistance genes. These genes information can be transferred vertically to the offspring or horizontally through mobile genetic elements (plasmids, prophages, integrons) [176,177].

The continuous mutations in the genome of microorganisms make it challenging to detect resistance genes. Short sequences of oligonucleotides (single-stranded DNA, PNA, LNA) immobilized onto transducer surfaces are the most common techniques in detecting ARGs by using electrochemical platforms, [47,86,178,179], SPR chip [180,181], or thin film transistor [182].

A direct gene-circuit/electrode interface for the electrochemical detection of colistin antibiotic resistance genes has been proposed by Mousavi et al. [183]. The colistin resistance genes (i.e., *mcr-1*, *mcr-2*, *mcr-3* and *mcr-4*) have recently been identified in livestock globally. The interesting strategy described in ref. [183] is based on the production of restriction-enzyme-based reporters to catalyze the release of a reporter DNA (single-stranded DNA, ssDNA, labeled with methylene blue), which hybridizes with a DNA capture probe immobilized onto the electrode surface. The approach begins with the synthesis of toehold switches complementary to the 24 top-ranked binding sites within each *mcr* gene and the ligation of a unique reporter enzyme gene to each set of switches. Restriction enzymes cleave annealed reporter DNA, which is free floating solution, releasing reporter DNA labeled with methylene blue. The capture probes then recruit the redox-active reporter DNA to the surface of nanostructured microelectrodes, generating an electrochemical signal. The approach was validated against the *mcr-4* gene. Thus, the *mcr-4* gene was expressed in *E. coli* and then total RNA was collected from the resulting culture. Selected RNA fragments were then amplified by isothermal amplification reaction (1 h) at a concentration of 30 ng/ μ L, using *mcr-4* specific primers. The amplified *mcr-4* mix was added without purification to solution containing the specific switch, followed by incubation off- and on-electrochemical chip for 45 min at 37 °C, prior to taking the first electrochemical measurement. The measurement was specific to *mcr-4* RNA in the presence of high background off-target RNA sequences and provided a strong, distinct signal against negative controls.

Korri-Yousoufi group [178,179] demonstrated the detection of *rpoB* gene resistance in *Mycobacterium tuberculosis* in real samples in a PCR-free approach. The sensitivity of the proposed assay was based on the association of magnetic nanoparticles and polypyrrole on a gold electrode surface. The catalytic properties of iron oxide nanoparticles combined with conducting properties of polypyrrole platform, allowed the detection of 1 fM of DNA target in a 50 μ L drop corresponding to 3×10^4 copies of DNA. The naphthoquinone redox signal decreased after the hybridization between a selected probe and the complementary DNA target without cross-hybridization with single nucleotide mismatch DNA target. The biosensors were applied to detect and discriminate wild type DNA from mutated DNA strands of *M. tuberculosis*, in both PCR amplified samples and samples without PCR amplification.

An electrochemical biosensor based on isothermal strand-displacement polymerization reaction for detection of *mecA* gene in methicillin resistance-*Staphylococcus aureus* (MRSA) was proposed in [184]. Methylene blue (MB)-labeled hairpin capture probes were immobilized by self-assembling on a gold electrode, in order to have the MB molecules confined close to the electrode surface for efficient electron transfer. After hybridization with the complementary target DNA, the hairpin probes undergo conformational changes, which led to a decreased electrochemical response. Furthermore, the primers annealed with the opened stems of the hairpin probes were extended by DNA polymerase, which in turn released the target DNA to trigger the next polymerase cycle. Therefore, a significant amplified current suppression for *mecA* gene detection is generated by the mass of MB molecules moved away from the electrode surface since many rounds can be performed.

Huang et al. developed an electrochemical biosensor based on a specific PNA probe to detect bla_{NDM}, the gene encoding the New Delhi metallo-beta-lactamase, using label-free EIS [185]. The capture probe for the single-stranded DNA of the resistance gene was immobilized on the surface of a gold screen-printed electrode. After extraction and digestion of the plasmatic DNA, hybridization with the biosensor was performed. The detection limit of 100 pM for PCR amplification products of the bla_{NDM} gene was obtained, with excellent results observed for amplification-free from a bla_{NDM}-harboring plasmid.

Rolling-circle amplification (RCA) was coupled to a simple electrochemical impedimetric sensor for the detection of resistance genes of antibiotic belonging to the diverse group of β -lactamases [186].

Using a bimodal waveguide interferometer (BiMW) technique, Maldonado et al. [187] demonstrated a direct detection, without amplification, of bla_{CTX-M-15} gene and bla_{NDM-5} gene, two clinically relevant and frequent antimicrobial resistance encoding sequences. Selected DNA capture probes were immobilized on the surface of the BiMW sensor chip. After DNA extraction, fragmentation, and denaturation process, the samples were carried out for alignment with the sensor (Figure 5). The BiMW biosensor showed high sensitivity (20–30 aM) and short analysis times (≤ 40 min, including sample pretreatment) for resistance gene analysis.

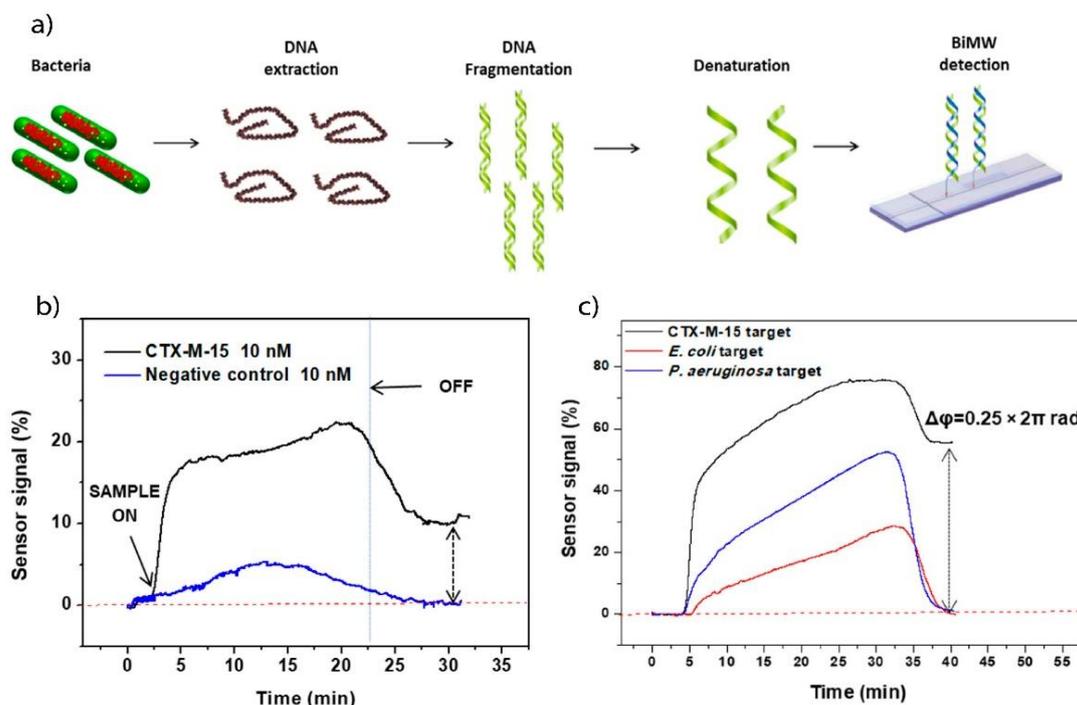


Figure 5. (a) Scheme of the biosensor strategy used for the detection of the genes; (b) signals obtained for the binding of the target (black line) and negative control (blue line) to the bla_{CTX-M-15}; (c) detection of bla_{CTX-M-15} gene (black line), non-specific *Pseudomonas aeruginosa* (blue line), and *Escherichia coli* without bla_{CTX-M-15} gene (red line). This article [187] is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>, accessed on 17 August 2021).

Hu et al. developed a system based on multiple cross displacement amplification (MCDA) assay and label-based lateral flow biosensor (LFB) for the amplification and detection of nucleic acids labeled with different chain displacement biomarkers in the presence of *Bst* polymerase under isothermal conditions [188]. In this work, the presence of the bla_{OXA-23-lik} (carbapenem resistance) gene was determined in *A. baumannii*. The reading of the results using the MCDA-LFB technique consists of capturing the amplification products on the test lines based on the combination of antibodies embedded in the LFB test lines and labeled antigens in the amplification products. The method shows a detection

limit of 100 fg per reaction with pure culture and high selectivity for the carbapenem resistance gene. Different types of genotypic sensors to determine resistance genes are shown in Table 5.

Table 5. Different genosensor for determination of antimicrobial resistance genes.

Technic	Recognition Element	Target	Type of Resistance	Limit of Detection	Previous Amplification	Reference
Electrochemical—EIS and CV	DNA probe	rpoB	Rifampicin resistance	0.08 fmol/L	Yes	[179]
Optic—fluorescence	DNA probe	CTX-MNDM-1	Cephalosporins resistance Carbapenems resistance	<10 copies of the gene	Yes	[182]
Optic—SERS	Hairpin-structured	tetA	Tetracycline Resistance	25 copies/ μ L	No	[189]
Optic—fluorescence	Fluorescent nucleic acid probe	VIM NDM IMP KPC	carbapenem antibiotic resistance genes	$(1.8 \pm 0.7) \times 10^6$ beads/mL per target	No	[190]
Electrochemical—EIS and CV	DNA probe	rpoB	Rifampicin resistance	0.2 fM	Yes	[191]
Optic—fluorescence	Binary deoxyribozyme	rpoB katG inhA	Rifampin resistance celsoniazid resistance Fluoroquinolone resistance	5 fg–15.6 pg	Yes	[192]
Electrochemical—EIS and CV	DNA probe	rpoB	Rifampicin resistance	0.1 fM–1 pM	No	[193]
Electrochemical—capacitance	DNA probe	ampR	Ampicillin resistance	1–4 pM	No	[194]
Electrochemical—DPV	DNA probe	MDR1	Multidrug resistance	2.95×10^{-12} M	No	[195]
Optic—fluorescence	DNA probe	rpoB	Rifampicin resistance	1 nM ssDNA in 1 mL sample volume	Yes	[196]
Optic—fluorescence	DNA probe	rpoB	Rifampicin resistance	100 nM	Yes	[197]
Optic—SPR	DNA probe	rpoB	Rifampicin resistance	NR	No	[198]
Electrochemical—DPV	PNA probe	rpoB	Rifampicin resistance	1 CFU/ml	No	[199]
Optic—fluorescence	Fluorescence DNA hairpin	mecR	Methicillin resistance	1 nM	Yes	[200]
Optic—fluorescence	Ab-DNA probe	lamB	Increases resistance to chlortetracycline, ciprofloxacin, balofloxacin and nalidixic acid	4–250 pM amplicon concentrations	Yes	[201]
Electrochemical—DPV	DNA probe	rpoB	Rifampicin resistance	20 fM	Yes	[202]
Mechanic—piezoelectric	DNA probe	mecR	Methicillin resistance	0.125 μ M	Yes	[203]

Ab, antibody; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; IMP, Imipenemase; KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo- β -lactamase; NR, not reported; SERS, surface-enhanced Raman spectroscopy; VIM, Verona integron metallo- β -lactamase.

3. Conclusions and Perspectives

The main challenges in AMR detection is to obtain reliable results in minutes or hours (instead of days as with standard techniques) and with easy-to-use and cost-effective tools.

Automated systems have helped in the pursuit to decrease analysis time in the deployment of AST and various methods based on optical imaging and microscopy-based techniques have been developed to measure bacterial growth [204–206].

Chemosensors and biosensors are one of the alternative techniques with the most significant projection in the coming years. The use of chemosensors and biosensors will result in the development of fast point-of-care devices in multiplexing formats. Progress in the different transducing techniques will allow AMR monitoring in low resource settings without the need for trained personnel.

However, despite the pressing market demands, commercially available tools are still at the development stage or do not meet validation requirements. There are a number of challenges that have to be surmounted in the validation of the developed methods. Key of which is the detection of low concentrations of analytes in the presence of high concentrations potential interferents. Enrichment and amplification are still necessary for the analysis of real samples due to the need to detect target species at low concentrations (i.e., initial concentration of bacteria in blood is around 1 to 100 CFU/mL) and eventually in the presence of a large excess of other bacterial species.

Integration of many different technologies is needed to solve problems related to low initial pathogen number and the presence of contaminating sample matrices. The problem of contamination can be avoided by the use of magnetic beads or nanoparticles coated with ligands-targeting bacteria. Furthermore, the development of novel isothermal amplification procedures will benefit (bio)sensor field. Among these techniques we can mention the nucleic acid sequence-based amplification (NASBA), the loop-mediated isothermal amplification (LAMP), the recombinase polymerase amplification (RPA), and others. These amplification methods have simplified nucleic acid amplification, often allowing a minimum sample treatment, cut down the costs of instrumentation and are suitable for miniaturization. Moreover, some of these techniques are less sensitive to inhibitors than standard PCR, allowing a minimal treatment of biological fluids. By contrast, multiplexing approaches are less developed for these isothermal amplification techniques than for PCR. Recently, clustered regularly interspaced short palindromic repeats (CRISPR)-associated methods have been studied for the detection of nucleic acids. This and other novel biochemical approaches represent innovative and powerful tools for rapid sensitive and selective monitoring of resistance genes.

Miniaturization of the transducers and the coupling with microfluidic platforms will enable the deployment of multiplexed, compact, and easy-to-use systems that will result in greater acceptance of chemosensors and biosensors by end-users and industrial stakeholders. Indeed, the progress in microfluidics, nucleic acid isothermal amplification, and (bio)sensor technology has recently provided several interesting devices that may eventually be used in AST, especially for low-resource field settings. An example is the Alveo platform (Alveo Technologies Inc, Alameda, CA, USA), an innovative device designed to multiplex 100 or more simultaneous tests from a single sample. The device consists of a fluidic cartridge coupled with electrochemical detection to measure the change in electrical conductivity that occurs during nucleic acid amplification: actually, during biochemical synthesis of DNA from nucleotides, the number and the mobility of electrically charged molecules are altered. Thus, the detection strategy is based on processing the sample by performing RPA and, optionally, a second isothermal amplification reaction. The second isothermal amplification reaction, which includes LAMP, can be carried out with amplification products of the RPA itself. The presence of the amplified target nucleic acid is then evaluated by measuring the impedimetric signal of the amplification reaction solution compared to a control. The analysis time can be of few minutes as well as many hours, depending on the desired degree of amplification that is necessary to achieve.

However, miniaturization requires a high level of standardization of the conditions, as the samples should represent similar growth states and culture densities. Automation can help with accurate reagent dispensing. The UtiMax Lab Automation System of Genefluidics Inc. (Duarte, CA, USA) is an automated diagnostic platform for the identification of

uropathogens directly from urine samples, consisting of a robotic liquid handling systems with associated reagent kits and disposable sensor array chips. As already mentioned the chip allows the electrochemical-measurement of species-specific ribosomal 16S rRNA. Each sample is lysed chemically. A built-in multi-channel potentiostat reads the electrical current from the steady-state enzymatic cycling amplification: signal is proportional to the bound 16S rRNA content from lysate and reported in ranges of CFU per milliliter through an established calibration curve.

Research in the field of chemo- and biomimetic receptors, including peptides, imprinted polymers and aptamers, will help in improving selectivity and thus bacterial identification that is another important issue for improving the reliability of the (bio)sensing approach to AMR monitoring. For instance, recently, antimicrobial peptides are considered as interesting biorecognition elements for bacteria ID and detection. These peptides can interact with bacteria, causing their lysis and inhibition of their growth. Some antimicrobial peptides have been described as specific to certain bacterial species, while other peptides can recognize any bacteria. This diversity can be employed both to design highly specific ligands and wide-spectrum probes able to interact with many different bacteria species. In the second case, profiling fingerprints and statistical analysis of data are necessary for the species-specific identification of bacteria.

Thus, in a context of growing global bacterial resistance, the development of better and more rapid detection of pathogenic threats based on biosensors is for sure one of the main interesting targets. This of course implies the development of more efficient sensing devices and also adequate biorecognition elements in the future to allow the specific identification of the various pathogens, particularly in medical settings where the nature of the threat is often not clearly identifiable.

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