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Study on the detection of antibiotics in food based on enzyme - free labelless aptamer sensor

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Abstract

The development of fast, specific, ultrasensitive, and accurate techniques for the early detection of important pathogens has been spurred by growing concerns about environmental safety, food safety, and nosocomial infection. Many bacteria, which may be found in foods, water, and medical equipment, cause diseases in humans and plants. In recent years, antibiotic resistance has arisen as a significant concern, as has their contamination as a result of unregulated use. As a result, there is a greater need to quickly establish reliable, simple, and sensitive techniques for evaluating antibiotics and their residues. The majority of bacterial detection procedures are lengthy and take days to provide a result. Aptamers appear to be a potential option for detecting bacteria in food items rapidly and reliably. Because of their high sensitivity, specificity, and selectivity, aptamer-based biosensors (aptasensors) attracted a lot of interest. This article provides a summary of newly discovered antibiotic detection aptasensors. This paper discusses the use of several aptamer assays to identify antibiotics such as sulfonamides, tetracyclines, lincosamide, (fluoro)quinolones, chloramphenicol, anthracyclines, aminoglycosides, and β-lactams.

Keywords: ampicillin; antibiotic; aptamer; aptasensor; biosensor.

Practical Application: The development of new analytical devices based on biosensors allowing high sensitivity and selectivity of antibiotic detection is of interest for the food industry, agricultural activities, public health protection. Currently, considerable attention is focused on the development of sensor devices that allow rapid determination of contaminants in the field and do not require highly skilled personnel. The study provides a practical contribution to the development of highly efficient analytical systems based on biosensors.

1 Introduction

Supervision over the quality and safety of food has tightened as customer worries about what's in their food and how it affects their health have grown, particularly in wealthy nations where food availability issues are much less of a problem. Indeed, the United States and the European Union also have to monitor programs with varying levels of coverage (Altunatmaz et al., 2017; Araújo et al., 2018). Since 2005, the EU has made it necessary to report suspected foodborne outbreaks. Despite this, there have been several significant foodborne epidemics in recent years. Antibiotic-resistant bacteria are becoming more common, posing a serious threat to public health and veterinary care (Gupta et al., 2019; Tacconelli et al., 2018). Antibiotic misuse and prophylaxis are prevalent (particularly in the field of animal welfare) and have been linked to metabolite and antibiotic pollution of the environment. This encourages bacteria to gain antibiotic resistance while it affects the ecosystem, for example, by causing excessive disruption of the ground flora. Antibiotic resistance can be reduced by detecting and limiting the use and discharge of antibiotics into the ecosystem.

Antibiotics are commonly occurring low molecular weight compounds produced by fungi or bacteria that destroy or inhibit the development of many other microorganisms. Antibiotics include chemically, and partly synthetic derivates produced substances with antibacterial activity in the broadest sense (Pang et al., 2019). In contemporary medicine, they are commonly employed to treat diseases. Antibiotics are divided into several categories based on their chemical composition and mechanism of action, including sulfonamides, lincosamides, tetracyclines, (fluoro)quinolones, anthracyclines, aminoglycosides, and β-lactams. Liquid chromatography-tandem mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) are now the most often used methods for detecting antibiotic residue levels in aqueous samples (Fu et al., 2019; Mehlhorn et al., 2018). Considering their vast variety of applications, these techniques are often time-consuming and need rigorous sample preparation, complex equipment, and skilled technical staff. The introduction of biosensors might solve these issues and allow for on-the-spot analysis.

A signal transduction element and a target identification component (e.g., cell, nucleic acid, enzyme, or protein) are two fundamental practical elements of biosensors (Adachi & Nakamura, 2019;

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Villalonga et al., 2020). Biosensors are classified as electrochemical-, optical-, or mass-based biosensors depending on their transducing element. Aptasensors, which are aptamer-based biosensors, have recently been created for antibiotic identification. Aptamers are single-stranded oligonucleotides (RNA or DNA) that could bind to a wide range of target molecules with high sensitivity, selectivity, and affinities such as metal ions, proteins, nucleic acids, and other tiny molecules (Han et al., 2010; Musumeci et al., 2017; Pandey & Qureshi, 2017). Aptamers are potential replacements to antibodies for most applications because of their advantages over antibodies. A technique known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX) can be used to find suitable aptamers (Ellington & Szostak, 1990; Stoltenburg et al., 2007). This technique allows the screening of nucleic acid aptamers (Aptamers) from a library of random single-stranded nucleic acid sequences with high affinity for the target material.

The basic idea of SELEX technology is to chemically synthesize a single-stranded oligonucleotide library in vitro, mix it with the target material, wash out the nucleic acid molecules that do not bind to the target material or have high affinity to the target material through repeated screening and amplification and isolate and purify the nucleic acid aptamers with high affinity to the target material. SELEX technology and nucleic acid aptamers have the advantages of large library capacity and wide range of adaptability, high resolution and practicality, high affinity, relatively simple, rapid, and economical screening process, and small aptamer size. The technology has applications in vitro diagnostics and in vivo therapeutic and drug development.

Appropriate binding sequences are first identified using this method and then amplified from vast oligonucleotide libraries. Aptamers can be easily changed with signal moieties and can be manufactured at a cheap cost. A range of assays and biosensors have been successfully created for aptamer-based analysis (detection and recognition) of various targets when the earliest aptamer-based biosensors were published (Davis et al., 1996; Drolet et al., 1996). Aptamer assays are being constructed as a dual-site (sandwich) binding format, a single-site binding format, in which a pair of aptamers sandwiched the analyte, or an aptamer and antibody are used in a sandwich binding configuration, similar to traditional immunosorbent assays. Aptasensors are made with transducers of many types that are electrochemically-, optically-, mass-sensitive. Figure 1 depicts the appropriate transduction principles (Bakker & Telting-Diaz, 2002; Clegg, 2009; Himmelstoß & Hirsch, 2019). A shift in an acoustic wave's oscillation frequency owing to a target binding is monitored in quartz crystal microbalance (QCM, Figure 1a); This technology is a mass-sensitive piezoelectric sensor that uses an aptamer as the sensing element and a quartz crystal chip as the transducer to convert the mass signal of the adsorbed material on the chip surface into a frequency signal for detecting the interaction between the aptamer and the target; and surface acoustic wave (SAW, Figure 1b) sensors.

Aptamer-target binding causes a shift in the microcantilever's frequency of resonance (dynamic mode) or steric crowding, which causes bending in the cantilever (static mode) in micromechanical cantilever arrays (MCA, Figure 1c). Optical or electrical detection is used to detect bending. The most popular optical biosensors employ fluorometric or colorimetric detection. Colorimetry is the process of determining a substance's concentration in a (mainly) fluid form by comparing it to a color spectrum that matches up to a recognized amount of substance. Because of their varied optical and electrical characteristics, colloid gold nanoparticles (AuNPs) were therefore widely used as a label for molecular identification. They efficiently accumulate and disperse light, are regarded as powerful quenchers, and come in a variety of hues. Localized surface plasmon resonance (LSPR), wherein electrons on the AuNPs interface simultaneously vibrate in sync with the incoming light, is responsible for the hues. AuNPs are catalytically active and have good biocompatibility, considerable surface free energy, and a wide surface area where molecules may be immobilized (e.g., aptamers).

The AuNPs have an aptamer linked to their surface and hence inhibit their aggregation in the colorimetric assay (CoA, Figure 1d) using gold nanoparticles. When the aptamer binds to its target, its conformation shifts to a folded rigid form from a random coil shape; as a result, the AuNPs and the adsorbed aptamers separate, and the AuNPs clump together. The solution's hue changes as a result of this. Colorimetric assays using AuNPs have a drawback that occurs when salt and other compounds are found in complicated biological liquids. AuNPs have a tendency to aggregate non-specifically. The aptamer is labeled with a suitable quencher and a fluorophore in the fluorometric assay (FlA, Figure 1e). When the target binds to the aptamer, the aptamer undergoes a conformational shift, bringing the quencher and fluorophore into close proximity, quenching the fluorescence. Signal-off mode is what it's called. The signal-on mode, in which the conformational shift caused by target binding causes the quencher and fluorophore to diverge, culminating in a fluorescence signal, is also conceivable. As a fluorescence monitoring platform, graphene oxide (GO) has been regularly used because of its great capabilities, minimal cytotoxicity, and superior biocompatibility for conjugation of target molecules, as a fluorescence monitoring platform, graphene oxide (GO) has been regularly used. Surface-modified graphene and GO are very effective fluorescence quenchers that work via energy or electron transfer processes.

The transmission of energy to a second chromophore (acceptor) from an excited chromophore (donor) without the use of radiation (dipole-dipole) is referred to as Förster resonance energy transfer (FRET). UCNPs (upconversion nanoparticles) are nanoscale particles that upconvert photons. This implies that photons are absorbed sequentially, resulting in light emission at wavelengths shorter than the excitation wavelength. UCNPs include quantum dots (QDs), which are semiconductor nanoparticles. Optical biosensors are used as substitutes to molecular fluorophores because of their influenceable optical and electrical characteristics. The underlying idea underpinning numerous lab-on-a-chip devices and biosensor applications for sensing chemical activity is the quantum mechanical phenomena of surface plasmon resonance (SPR, Figure 1f). SPR is a flexible method that does not require any extensive sample processing or radioactive or enzyme-labeled reagents. Surface-enhanced Raman scattering (SERS, Figure 1g) is a method that boosts Raman intensity by orders of magnitude, overcoming Raman scattering's conventional flaw - its intrinsic weakness.

The following is the basic concept of an aptamer-based electrochemical biosensor (Figure 1h): Fe_3O_4 NPs, methylene

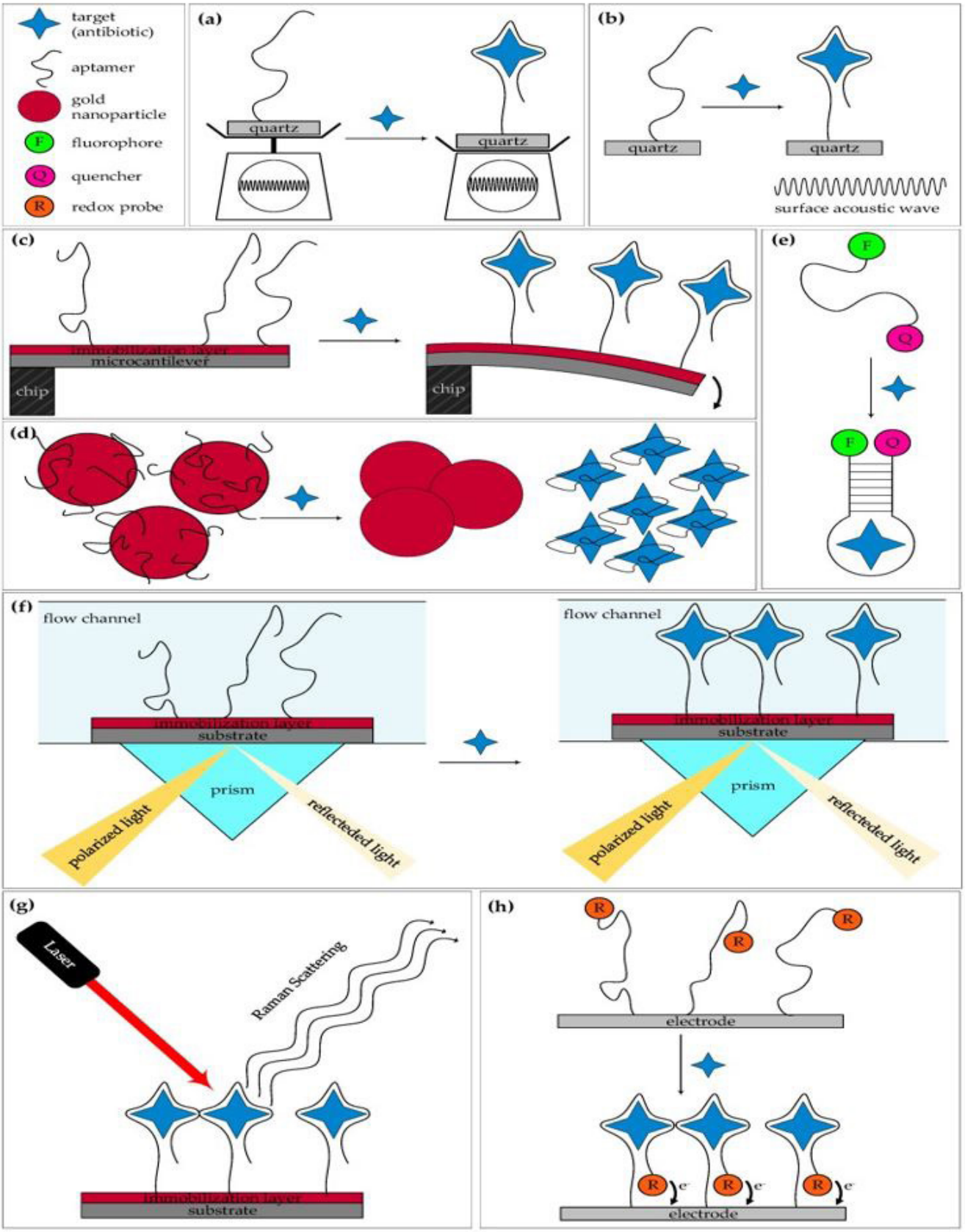

Figure 1. The most often utilized aptasensors' working principles. (a) Quartz crystal microbalance; (b) surface acoustic wave; (c) micromechanical cantilever array; (d) AuNPs based colorimetric aptasensor; (e) fluorometric aptasensor; (f) surface plasmon resonance; (g) surface-enhanced Raman scattering; and (h) electrochemical aptasensor.

blue (MB), or a redox probe (typically ferrocene) are used to immobilize the aptamer onto an electrode surface. When the aptamer binds to its target, it changes conformation, bringing the electrode surface closer to the probe (Bakker & Telting-Diaz, 2002; Himmelstoß & Hirsch, 2019). The signal on mode allows for the flow of electrons and hence an electrochemical readout. On the other hand, the conformational shift raises the space between the surface electrode and the redox probe, interrupting the prior electron transport, which is known as signal off mode. Using different metal ions with different redox potentials to create distinct electrochemical signals, such as Cu^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+}, it is feasible to identify more than one target analyte at the same time. Metal-labeled bio codes are the name given to these types of probes. The measurement principle distinguishes four different types of electrochemical sensors (Crow, 2017):

- Amperometric that detects the change in current potentials whenever pairing arises throughout redox processes;
- Conductometric that detects changes in electrical charge in a solution at a steady voltage;
- Impedimetric that detects impedance variations as a result of the interaction.
- Potentiometric, that detects variations in the potential electrical discrepancy when two objects are bound together;

The use of aptamers in the identification of various antibiotic classes is discussed in this thorough and comprehensive study.

2 Aptasensors for various classes of antibiotic

The many recently produced biosensors based on aptamers for antibiotic detection that have been referenced in the literature are listed and described here, organized by antibiotic class. The following criteria or qualities are critical for comparing the performance of aptasensors (Daprà et al., 2013; Gurtu et al., 1997; Lan et al., 2017):

Affinity is a measure of a molecule's proclivity to attach to another one. The association constant K_A increases as the affinity increases. The dissociation constant K_D, which has a reciprocal value, is more frequent. Low K_D values are desirable since the stronger a target's affinity for its ligand, the lower the complex's K_D.

Selectivity refers to the ability to choose numerous things from a collection of options, whereas specificity refers to the ability to choose one object from a group of options. When distinct elements of a combination are identified without interference, side by side, an analytical approach is selective. When just one component of a combination can be identified, the approach is specific. It is preferable to have a high level of selectivity and specificity.

Within a certain confidence limit, the limit of detection (LOD) is the smallest amount of a chemical that can be differentiated from its absence (a blank value). Maximum residue levels (MRL) are the greatest concentrations of an unwanted chemical (pollutant or impurity) that are lawfully allowed in a commodity or food, as established by the European Union, for example, residues of antibiotics in animal products and live animals are regulated under European Council Directive 96/23/EC. The goal of biosensor development is to achieve a decreased level of sensitivity where the MRL is more than the LOD. The repetition of scientific study findings is known as reproducibility.

Using a conventional addition approach, the recovery is calculated. The recovery of defined target concentrations is detected after they are applied to actual samples. The outcomes are also compared to those obtained using a different approach, the enzyme-linked immunosorbent assay (ELISA).

Identifying the target in actual samples like water, serum, milk, honey, etc., demonstrates the usefulness of the developed aptasensor for real-sample analysis.

A sensor's stability refers to its capacity to keep performing under the same circumstances for an extended length of time. It is put to the test by keeping the sensor for several weeks under specific conditions and evaluating prior to and after preservation of analytical findings.

2.1 β-Lactams

β-lactams antibiotics, in terms of value and quantity, are the most significant antibiotics due to their low toxicity, great effectiveness, and ability to derivatize them using enzymatic and chemical techniques. The mechanism through which they work is the inhibition of cross-linking of peptides in the bacterial cell wall (murein). Their β-lactam ring serves as a distinguishing feature (outlined in red in Figure 2) (Majdinasab et al., 2019; Sun et al., 2021).

The applied sensor type, real sample analysis (RSA), the limit of detection (LOD), and dissociation constant (K_D) are summarized in Table 1 and Table 2 for the mentioned aptasensors for β-lactams.

Ampicillin

Penicillin-resistant strains have prompted researchers to look for other antibiotics, leading to the discovery of semi-synthetic penicillins like ampicillin (Figure 2a). With the help of nicking endonuclease and polymerase, two electrochemical aptasensors were devised to improve the aptasensor responsiveness to ampicillin as well as signal amplification.

Penicillin

The structure of penicillin potassium salt is modified by chemical and enzymatic methods to obtain different intermediates, such as 6-aminopenicillins acid (6-APA), which is made from penicillin G (Figure 2b). Tetanus, angina, gonorrhea, diphtheria, and scarlet fever are among the most common bacterial diseases treated with lactam antibiotics. The fungi Penicillium notatum is used to make Penicillin, and it is seldom poisonous to humans. Despite the fact that penicillin is a commonly used antibiotic, just a few aptamer-based biosensors can detect it. As a result, more study is still possible.

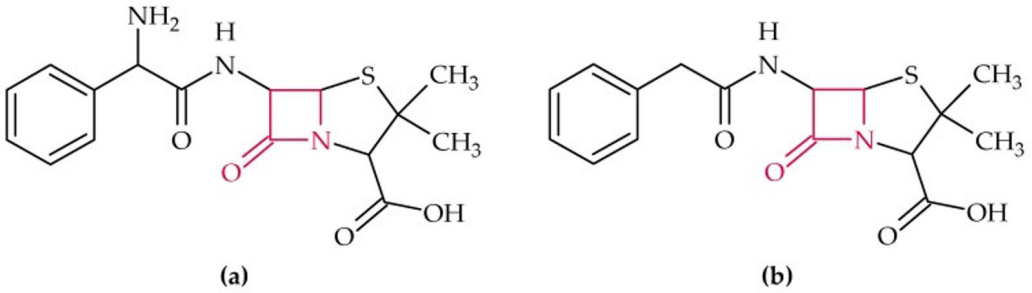

Figure 2. Chemical structure in (a) ampicillin and (b) penicillin G. Outlined in red is the β-lactam ring.

Table 1 - Aptasensor characteristics for detection of ampicillin.

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
AEC/AC, SWV	bsa, sa, m	1000 (AC) 30,000 (SWV)		MB	SH-$(CH_2)_6$
AEC/AC	hu, w, m, sa	30 (b)	-	MB	SH-$(CH_2)_6$
IEC/EIS	-	0.1 (b)	-	-	poly(T)-poly(C)
IEC/EIS	m	0.1 (b)	13.4	-	NH_2-C_6
AEC/DPV	m	0.004 (b)	-	A_{10}	MB
FL, CO/UV–VIS	m	I: 1.4 (dw, FL) I: 5.7 (m, FL) I: 14.3 (dw, CO) I: 28.6 (m, CO)	I: 13.4 II: 9.8 III: 9:4	-	FAM
AEC/DPV	m	0.00038 (b)	-	-	apt: SH cDNA: SH
EBFC/CV, OCV	m	0.003 (b)	-	-	apt: NH_2-$(CH_2)_6$ cDNA: SH-$(CH_2)_6$
FL	rw	0.2 (b) (0.07×10^{-6} g/L)	-	apt I: SH apt II:	-
AEC/DPV	m	0.001 (b)	-	-	-

w = water, SWV = square wave voltammetry, sa = salvia, rw = river water, real sample analysis (RSA), OCV = open circuit voltage, MB = methylene blue, m = milk, limit of detection (LOD), IEC = impedimetric electrochemical, hu = human urine, FL = fluorometric, FAM = fluorescein amidite, EIS = electrochemical impedance spectrometry, EBFC = enzyme biofuel cell, DPV = differential pulse voltammetry, dissociation constant (K_D), CV = cyclic voltammetry, CO = colorimetric, cDNA = complementary DNA, bsa = bovine serum albumin, apt = aptamer, AEC = amperometric electrochemical, and AC = alternating current.

Table 2. Aptasensor characteristics for detection of penicillin.

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
FL	m	9.2 (b)	383.4	-	FAM
IEC/EIS	m	0.49 (b) (0.17×10^{-6} g/L)	-	-	-
IEC/EIS	m	0.057 (b)	-	-	NH_2

real sample analysis (RSA), limit of detection (LOD), IEC = impedimetric electrochemical, FL = fluorometric, FAM = fluorescein amidite, EIS = electrochemical impedance spectrometry, dissociation constant (K_D), and b = buffer.

2.2 Aminoglycosides

Antibiotics that include aminoglycosides are by far the most commonly used antibiotics in the world, with a wide range of action that includes Gram-negative bacteria. They are the antibiotics of choice for serious infections, despite their somewhat high toxicity (esp. on the kidneys and ears), which contributes to an escalation in resistance. An aminocyclitol ring is included in most aminoglycoside antibiotics that are glycosidically connected to other amino sugars as their fundamental structure (Figure 3) (Fisher & Mobashery, 2021; Rapini & Marrazza, 2017). Tables 3-7 highlight the most significant facts for the aminoglycosides mentioned aptasensors, comprising sensor type, RSA, LOD, and K_D used (Ball et al., 1978; Becker & Cooper, 2013; Parmar et al., 2000).

Gentamicin

Gentamicin is a wide-ranging antibiotic that works as a bactericide against a diverse set of Gram-positive/negative bacteria, including Pseudomonas aeruginosa and E. coli (Figure 3a). It was

first identified in 1963. An electrochemical aptasensor based on DNA/RNA aptamers has been designed to detect kanamycin, tobramycin, and the aminoglycoside medicines gentamicin in blood samples, preventing side effects and overdosage (Rowe et al., 2010).

Kanamycin

Streptomyces kanamyceticus produces kanamycin, a bactericidal antibiotic that kills Gram-negative and Gram-positive bacteria alike (Figure 3b). A lot of research is focused on kanamycin aptamer-based sensors because selective and sensitive techniques to detect kanamycin residues for clinical diagnosis and safety of food are of considerable importance (Tang et al., 2018).

Neomycin

The majority of Gram-negative bacteria, such as Shigella and Salmonella, are susceptible to neomycin (Figure 3c) (Nikolaus & Strehlitz, 2014).

Tobramycin

Aminoglycoside antibiotics (e.g., tobramycin) are semi-synthetic and have a long half-life (Figure 3d). Numerous

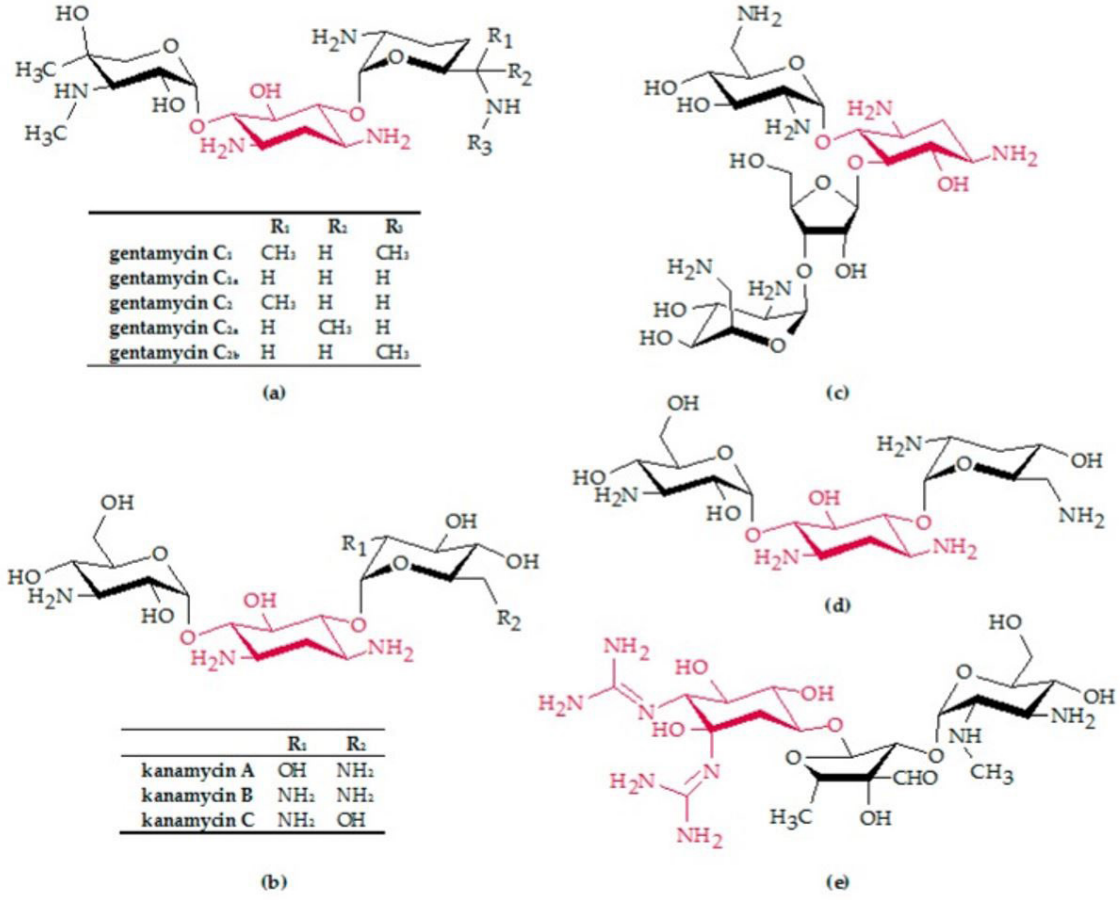

Figure 3. Chemical make-up of: (a) gentamicin; (b) kanamycin; (c) neomycin B; (d) tobramycin; and (e) streptomycin. Shown in red are aminocyclitol rings which are connected to other amino sugars glycosidically in the fundamental structure of aminoglycoside antibiotics.

Table 3. Aptasensor characteristics for detection of gentamicin.

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
AEC/SWV	hs	-	I: 72,000 II: ≈ 80,000 III: ≈ 200,000	I: NH-MB II: NH-MB III: NH-MB	I: SH II: SH III: SH

SWV = square wave voltammetry, real sample analysis (RSA), MB = methylene blue, the limit of detection (LOD), hs = human serum, for gentamicin, a sensor type, and measurement method have been created, dissociation constant (K_D), and AEC = amperometric electrochemical.

Table 4. Aptasensor characteristics for detection of kanamycin.

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
ECL	m	0.045 (b)	-	-	SH-$(CH_2)_6$-T_5
CAN	-	50000 (b)	-	-	SH-$(CH_2)_6$
CO/UV–VIS	m	0.014 (b)	-	-	SH-$(CH_2)_6$
PEC/EIS, CA	-	0.1 (b)	-	-	SH-$(CH_2)_6$
PEC/EIS	m	7.2 (b) (3.5×10^{-6} g/L)	-	-	SH-$(CH_2)_6$
AEC/DPV	m	0.00137 (b) (0.008×10^{-9} g/L)	-	-	SH
AEC/DPV	m, p, c	0.87 (b)		-	PO_4
FL	m	1100 (b)	-	-	NH_2-C_6
IEC/EIS	m	10 (b)	78.8	-	NH_2-C_6
FL	bs	0.009 (b) 0.018 (bs)	-	-	NH_2
AEC/SWV	m	9.4 ± 0.4 (b) 10.8 ± 0.6 (m)	78.8	-	NH_2
AEC/DPV	m	0.0037 (b)	-	-	NH_2
AEC/DPV	m, p, c	0.00042 (b)	-	-	NH_2
FL	m, bs	I: 6.25 (b) II: 6.25 (b) II: 0.001 (st) II: 0.1 (bs) II: 0.02 (m)	II: 92.3	-	II: FAM
AEC/SWV	hs	-	I: 281,000 II: ≈ 450,000 III: ≈ 600,000	I: NH-MB II: NH-MB III: NH-MB	I: SH II: SH III: SH
AEC/DPV	m	5.8 (b)	-	-	I: NH_2 II: biotin
AEC/DPV	m	8.6 (b)	-	-	I NH_2 II biotin
FL	ww	5000 (ww)	3900	-	FAM
FL	m	0.3 (b)	115 ± 2.76	-	FAM
IEC/EIS	lw	1.0 (b)	78.8	apt I: $(CH_2)_3$-SH apt II: $(CH_2)_3$-ferrocene	cDNA I: ferrocene-$(CH_2)_6$ cDNA II: SH-$(CH_2)_6$
AEC/DPV	m	7.9 (b) ($4{,}6 \times 10^{-6}$ g/L)	-	-	biotin
AEC/SWV	m	0.00016 (b)	-	-	apt: NH_2-$(CH_2)_6$ cDNA: NH_2-$(CH_2)_6$
ECL	m	0.034 (b) (0.002×10^{-6} g/L)	-	-	apt: biotin cDNA: SH-$(CH_2)_6$
CO	m, h	0.0778 (b)		apt: biotin	apt: SH-$(CH_2)_6$ cDNA I: SH-$(CH_2)_6$ cap: biotin
AEC/SWV	m	0.01 (b) (0.005×10^{-6} g/L)	-	-	apt: SH cDNA: apt
FL	m, h, p	1.58 (b) (0.92×10^{-6} g/L)	-	-	apt: biotin cDNA: ROX
FL	rs	0.612 (b) 0.453 (rs)	-	-	apt: biotin cDNA: FAM
FL	m	26 (b)		apt IV: Cy3 cDNA I: Cy3	apt II: Cy3 apt III: Cy5 anchor apt: NH_2 cDNA II: Cy3
AEC/SWV	m	0.000035 (b)			apt I: SH
FL	m	0.4 (b)	-	cDNA I: FAM cDNA II: FAM cDNA III: FAM	apt I: FAM

ww = waste water, SWV = square wave voltammetry, ROX = 6-carboxyl-x-rhodamine, rs = rat serum, real sample analysis (RSA), PEC = photoelectrochemical, p = pork, MB = methylene blue, m = milk, lw = lake water, LCA = liquid crystal assay, limit of detection (LOD), IEC = impedimetric electrochemical, hs = human serum, for kanamycin, a sensor type and measurement method have been developed, FL = fluorometric, FAM = fluorescein amidite, f = fish, EIS = electrochemical impedance spectrometry, ECL = electrochemiluminescent, DPV = differential pulse voltammetry, dissociation constant (K_D), cy = cyanine dye, CO = colorimetric, cDNA = complementary DNA, cap = capture probe, CAN = cantilever, CA = chronoamperometry, c = chicken, b = buffer, bs = blood serum, apt = aptamer, and AEC = amperometric electrochemical.

Table 4. Continued...

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
CO/UV–VIS	-	25 (b)	78.8	-	-
CO/UV–VIS	-	-	78.8	-	-
CO	-	1.49 (b)	8.38	-	-
CO	m	4.5 (b) (2.6×10^{-6} g/L)	-	-	-
CO	m	3.35 (b)	-	-	-
LCA	-	<1 (b)	-	NH_2-$(CH_2)_6$	-
FL	m, rs	0.321 (b) 0.476 (m) 0.568 (rs)	-	-	-
FL	m	59 (b)	-	-	-
ECL	f	143 (b)	78.8	-	-
PEC	-	0.2 (b)	-	-	-
IEC/EIS	m	1.0 (b)	-	-	-
IEC/EIS	m	0.23 (0.11×10^{-6} g/L)	-	-	-
AEC/SWV	m	0.00015 (b)	78.8	$(CH_2)_6$-NH_2	-
AEC/DPV	m	0.00074 (b)	-	-	-
AEC/DPV	m	0.0000013 (b)	-	-	-
FL	m	0.3 (b)	-	-	-

ww = waste water, SWV = square wave voltammetry, ROX = 6-carboxyl-x-rhodamine, rs = rat serum, real sample analysis (RSA), PEC = photoelectrochemical, p = pork, MB = methylene blue, m = milk, lw = lake water, LCA = liquid crystal assay, limit of detection (LOD), IEC = impedimetric electrochemical, hs = human serum, for kanamycin, a sensor type and measurement method have been developed, FL = fluorometric, FAM = fluorescein amidite, f = fish, EIS = electrochemical impedance spectrometry, ECL = electrochemiluminescent, DPV = differential pulse voltammetry, dissociation constant (K_D), cy = cyanine dye, CO = colorimetric, cDNA = complementary DNA, cap = capture probe, CAN = cantilever, CA = chronoamperometry, c = chicken, b = buffer, bs = blood serum, apt = aptamer, and AEC = amperometric electrochemical.

Table 5. Aptasensor characteristics for detection of neomycin.

Sensor Type/Method	RSA	K_D (nM)	LOD (nM)	3′ Linker and Spacer	5′ Linker and Spacer
FL	m	115 ± 25	10 (m)	$(T)_{15}$–$(A)_{12}$	FAM
IEC/FIS	m	-	<1000 (b)	-	-
IEC/FIS SPR	-	2500 ± 900	5 (b, SPR)	-	-

SPR = surface plasmon resonance spectroscopy, real sample analysis (RSA), m = milk, limit of detection (LOD), IEC = impedimetric electrochemical, for neomycin B, a sensor type and measurement method have been developed, FL = fluorometric, FIS = Faradaic impedance spectroscopy, FAM = fluorescein amidite, dissociation constant (K_D), and b = buffer.

Table 6. Aptasensor characteristics for detection of tobramycin.

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
SPR	bs	500 (b) 3400 (bs)	260	-	SH
AEC/SWV	bsa	-	I: 16,000 ± 3000 II: 220 ± 50 III: 510 ± 70 IV: 2900 ± 900 III: 148,000 ± 4000 (s)	I: MB II: MB III: MB IV: MB	I: SH-C_6 II: SH-C_6 III: SH-C_6 IV: SH-C_6
AEC/SWV	hs	-	I: 319,000 II: ≈ 180,000 III: ≈ 1,380,000	I: NH-MB II: NH-MB III: NH-MB	I: SH II: SH III: SH
AEC/DPV, CA	hs	100 (b)	-	-	fluorescein
AEC/DPV	-	5000 (b)	-	-	biotin
CO	h	37.9 (b)	I: 56.9 II: 46.8 III: 48.4	-	-
CO	m, ce	23.3 (b)	-	-	-
IEC/FIS	hs	I: 700 (b) II: 400 (b)	I: 600 II: 400	-	-

Tobramycin sensor type and measurement method have been realized, SWV = square wave voltammetry, SPR = surface plasmon resonance, real sample analysis (RSA), MB = methylene blue, m = milk, limit of detection (LOD), IEC = impedimetric electrochemical, hs = human serum, h = honey, FIS = Faradaic impedance spectroscopy, DPV = differential pulse voltammetry, dissociation constant (K_D), CO = colorimetric, ce = chicken egg, CA = chronoamperometry, bsa = bovine serum, albumin, bs = blood serum, b = buffer, and AEC = amperometric electrochemical.

Table 7. Aptasensor characteristics for detection of streptomycin.

Sensor Type/Method	RSA	LOD (nM)	K_D (nM)	3′ Linker and Spacer	5′ Linker and Spacer
CO	m	0.0017 (b) ($1{\cdot}10^{-9}$ g/L)	-	-	SH-$(CH_2)_6$
AEC/SWV	m	10 (b)	-	cDNA II: biotin	cap: SH-$(CH_2)_6$
CO/UV–VIS	h	I: 200 (b) I: 200 (h)	I: 199.1 II: 221.3 III: 272.0 IV: 340.6	-	-
CO	m, h	25 (b)	I: 6.07 II: 8.56 III: 13.14	I: biotin II: biotin III: biotin	I: FAM II: FAM III: FAM
CO	m	86 (b)	199.1	-	SH
CO, FL/UV–VIS	m, bs	73.1 (b, CO) 102.4 (bs, CO) 108.7 (m, CO) 47.6 (b, FL) 58.2 (bs, FL) 56.2 (m, FL)	-	-	cDNA: FAM
FL	m, bs	54.5 (b) 71.0 (rs) 76.05 (m)	-	-	-
FL	m	0.05 (b)	-	-	-
FL	m, c	94 (b)	-	-	-
PEC	h	0.033 (b)	-	NH_2	-
IEC	hs	$0.057{\cdot}10^{-3}$ (b) ($0.033{\cdot}10^{-9}$ g/L)	-	SH	-
AEC/DPV	m, rs	11.4 (b) 14.1 (m) 15.3 (rs)	-	SH	-
AEC/DPV	m, h	0.036 (b)	-	SH	-
AEC/DPV	m	0.0078 (b)		-	NH_2
AEC/DPV	m	0.028 (b)	-	SH	-

SWV = square wave voltammetry, streptomycin sensor type and measurement technique have been realized, rs = rat serum, real sample analysis (RSA), PEC = photoelectrochemical, m = milk, limit of detection (LOD), IEC = impedimetric electrochemical, h = honey, FL = fluorometric, FAM = fluorescein amidite, DPV = differential pulse voltammetry, dissociation constant (K_D), CO = colorimetric, cDNA = complementary DNA, cap = capture probe, bs = blood serum, b = buffer, and AEC = amperometric electrochemical.

Gram-negative pathogens, including Shigella, Salmonella, Pseudomonas, Proteus, Klebsiella, and E. coli, as well as Gram-positive Enterococci and Staphylococci, are included in its spectrum of activity (Luan et al., 2020).

Streptomycin

Selmon Waksman's Streptomycin's discovery (Figure 3e) in 1943 enabled the TB pathogen Mycobacterium tuberculosis to be cured for the first time (Li et al., 2015). Today, however, alternative antibiotics are commonly employed due to streptomycin's renal and ear-harming characteristics.

3 Conclusion

The creation of extremely rapid, sensitive, and precise analytical methods for water and food analysis has been spurred by global water and food safety concerns. Because of its unique mix of benefits, the electrochemical aptamer-based detection platform (E-aptasensor) is one of the most promising detection approaches. These sensors are excellent for the detection of a wide range of target analytes. Foodborne illnesses are a major public health concern across the world. Countless individuals fall sick, and some even perish each year owing to consuming contaminated water and food. The situation is made much more complicated by the so-called globalization of food delivery, processing, and production. Antibiotic resistance has developed as a major concern owing to the unregulated and extensive use of antibiotics in recent years.

As a result, on-site screening analysis requires robust, sensitive, simple, and quick methodologies for evaluating antibiotics and their residues. Instrumental techniques, such as liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE), as well as methods combined with mass spectrometry, are the most often used traditional techniques for antibiotic identification (LC-MS). Considering their broad variety of functionalities, these technologies typically have drawbacks, such as the need for expensive laboratory gear, trained workers, and lengthy separation/sample processing procedures. Because of its advantages, such as fast detection, excellent selectivity, and in situ usage, biosensors are regarded as suitable choices for detecting antibiotics. As a result, biosensor research and the production of novel signal transduction

systems is a fast-expanding subject in environmental, clinical, and biological sciences.

Aptamer-based biosensors (aptasensors) are one type of biosensor that has a lot of promise. Electrochemical aptamer biosensors are the most commonly employed for antibiotic detection because of its low cost, mobility, high sensitivity, and operational simplicity. Oxytetracycline, tetracycline, chloramphenicol, and Kanamycin are among the most widely studied antibiotics detected by an aptasensor. As a result, there is a lot of opportunity for creating high-affinity and specificity aptamers for additional antibiotics. Finally, aptamer-based sensors have the ability for point-of-care detection as well as clinical applications, which is one of the benefits of aptamers over antibodies.

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