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Rapid antibiotic biosensors based on multiple molecular recognition elements

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The extensive use of antibiotics poses significant public health concerns, including the increase in drug-resistant bacteria and environmental pollution, underscoring the urgent need for rapid, sensitive, and specific antibiotic detection methods. Most current reviews on antibiotic detection primarily focus on categorizing antibiotics based on their types or the classification of sensors used, such as electrochemical, optical, or colorimetric sensors. In contrast, this review proposes a novel and systematic theoretical framework for the detection of antibiotics using sensors using seven popular molecular recognition elements-antibodies, aptamers, microorganisms, cells, peptides, molecularly imprinted polymers (MIPs), metal–organic frameworks (MOFs) and direct recognition modalities and briefly discusses the mechanism of molecular recognition elements and antibiotic recognition. Additionally, it explores biosensors developed using these elements, offering a detailed analysis of their strengths and limitations in terms of sensitivity, specificity, and practicality. The review concludes by addressing current challenges and future directions, providing a comprehensive perspective essential for enhancing food safety and protecting public health.

1. Introduction

Since Alexander Fleming discovered penicillin in 1929, antibiotics have played an indispensable role in medicine, agriculture, and livestock, gradually becoming a major revolution in human history. Based on their chemical structure, antibiotics can be broadly classified into several categories: quinolones, β-lactams, macrolides, aminoglycosides, sulfonamides, tetracyclines, nitroimidazoles, and lincosamides.[1]

Studies have identified trace amounts, even at the nanogram or microgram level, of antibiotic residues in commonly consumed foods such as pork, milk, and fish, a phenomenon that is alarmingly prevalent.[2] Research conducted by Zhang and Wang *et al.* has detected antibiotics such as tetracycline (TC) and oxytetracycline (OTC) in the water of Honghu, primarily stemming from the antibiotics used in aquaculture.[3] The residue of these antibiotics can increase bacterial resistance.[4] The excessive use of antibiotics can even impact the survival of aquatic organisms. In the medical realm, the appropriateness of antibiotic usage is closely linked to patient health, as it aids in alleviating suffering and significantly reduces mortality rates caused by bacterial infections, thus transforming previously deadly diseases such as pneumonia[5] and tuberculosis[6] making the transformation controllable. Excessive use of antibiotics can lead to the development of antimicrobial resistance to commonly used medications.[7] Therefore, the dosage of antibiotics is critical. Additionally, due to the global consumption of antibiotics and incomplete metabolism,[8] gradually, various issues have arisen due to antibiotic residues (MRL) and the resistance they induce.[9,10] For instance, nephrotoxicity, ototoxicity, and allergic reactions, among other detrimental effects, warrant increased attention from the scientific community. According to data from the World Health Organization (WHO), it is projected that by 2050, antibiotic resistance will result in a minimum of 10 million fatalities.[11] The European Union has banned the use of certain antibiotics, making it necessary to develop effective methodologies for the efficient and rapid detection of antibiotic residues. There is an urgent need to create highly sensitive and low-cost detection methods, as this is a critical pathway for advancing contemporary science.

Molecular recognition relies on specific recognition elements or distinct target properties, such as charge ratios or mass-to-charge ratios.[12] They play a critical role in fields such as biology, chemical analysis, and sensor technology. These elements interact with target molecules through various intermolecular forces, including hydrogen bonding, electrostatic interactions, hydrophobic interactions, π–π stacking, and van

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der Waals forces.[13] These elements can form stable complexes with specific target molecules, enabling the recognition and detection of those molecules. Currently, common recognition elements for antibiotics include antibodies, aptamers, microorganisms, cells, peptides, molecularly imprinted polymers (MIPs), and metal–organic frameworks (MOFs). In addition, certain instruments and devices allow direct identification of antibiotics from their own molecular state, as their molecular structure and functional groups determine their specific mass spectral fragments and peaks.

Biosensors, which integrate biological recognition elements with physical detection devices, are analytical tools capable of converting biological signals into measurable physicochemical signals such as electrical or optical signals. Typically, a biosensor consists of two main components: a molecular recognition element and a transducer.[14] Biosensors can be classified based on signal type into categories such as electrochemical, optical, fluorescence, and colorimetric sensors, all of which are widely used in the field of antibiotic detection today.

This article reviews various molecular recognition elements currently used for antibiotic detection, including antibodies, aptamers, microorganisms, cells, peptides, MOFs, MIPs and direct recognition modalities. It also covers biosensors based on these recognition elements and provides a brief summary of the recognition mechanisms of the two most commonly used elements, antibodies and aptamers, in antibiotic detection. Finally, the future trends for each type of molecular recognition element are anticipated (Fig. 1). The review provides new ideas and theoretical references in the field of antibiotic detection in different environments.

2. Recognition mechanism of molecular elements and antibiotics

Molecular forces are essential for the tight binding of various substances in the biological world, whether they are large or small molecules. The five molecular interactions in this review, hydrogen bonds, electrostatic interactions, π–π stacking, van der Waals forces, and hydrophobic interactions, are commonly occurring molecular forces. Among them, hydrogen bonds and van der Waals forces are covalent interactions, while the other three forces are non-covalent interactions.[15] They are tightly

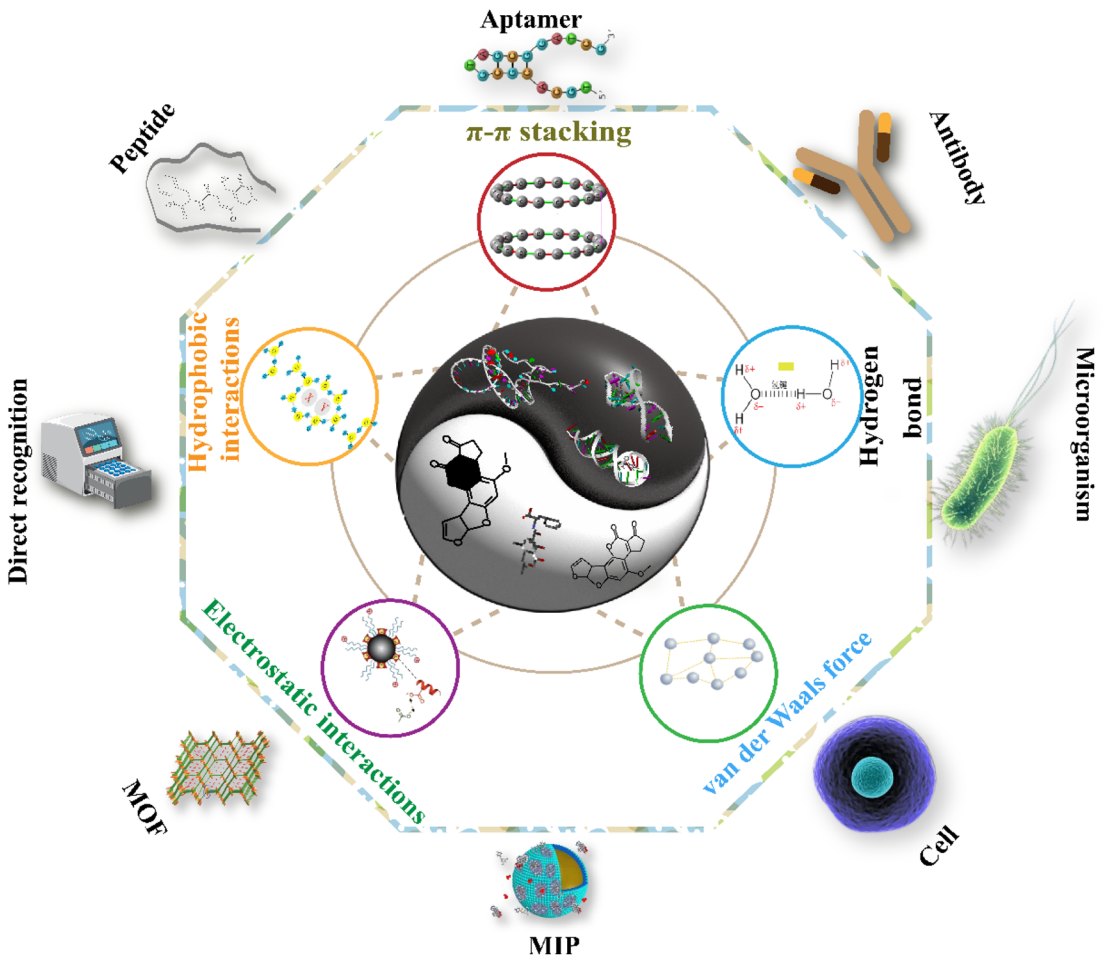

Fig. 1 The molecular recognition elements utilized for antibiotic detection, along with the interactions between them. In this framework, the inner circle represents the five types of intermolecular forces, while the outer circle embodies the seven molecular recognition elements and direct recognition.

bound to different molecular recognition elements through covalent or non-covalent forms, and also recognition elements need to have excellent affinity and specificity.

Aptamers, antibodies, and peptides act in a similar way, so they usually bind to antibiotics with nearly the same molecular force. Zhu and Liu *et al.* developed a fluorescent probe for the detection of antibiotics in glycopeptides.[16] Summarizing the results of previous studies, peptide amino acid sequence D-Ala-D-Ala and glycopeptide antibiotics are bound by hydrogen bonds to the sequence at each end of each fluorescence and quenching groups; the two group pairs involves a large hydrophobic force, which avoids the interference of other substances. Chaudhari *et al.* developed a fluorescent iron carrier pyoverdine (PVD) from bacteria, and they probed the interaction of PVD and ciprofloxacin using fluorescence spectroscopy and found that it encapsulates ciprofloxacin through the hydrogen bond, which is measured in a 1 : 1 ratio and leads to a change in the final fluorescence signal.[17] In addition, the recognition elements are detected by their electrostatic interactions using a number of fluorescent dyes.[18]

As for MIPs and MOFs, the two tend to immobilize antibiotics to their surfaces through π–π stacking or some other molecular interaction forces. Wang and Liu *et al.* developed two porous crystalline materials, COF-1 and COF-2, for the detection of antibiotics in water which can be quenched by π–π stacking as fluorescence due to the fact that antibiotics contain a benzene ring, which gives them the ability to detect antibiotics.[19] For the identification of various molecular forces, they can be simulated and predicted using instruments, such as computer simulations, molecular docking, and single crystal X-rays.

3. Antibody-based detection of antibiotics

3.1 Antibody–antibiotics recognition mechanism

Antibodies are proteins produced by B cells in the immune system that can specifically recognize and bind to particular antigens, including proteins and small molecular compounds. Antigen-binding sites are determined by the arrangement of amino acids and have a unique spatial structure that specifically recognizes antibiotic molecules.[20] The amino acid sequences in the variable regions of antibodies can bind to specific functional groups. Through screening and optimization processes, antibodies with high affinity for antibiotics can be obtained, resulting in highly specific recognition. Leivo *et al.* engineered antibodies to simultaneously detect eight fluoroquinolone antibiotics. They utilized a high-mutation-efficiency library and, after a single round of screening, calculated the binding affinities using time-resolved fluorescence immunoassays. The binding variations among the fluoroquinolones (FQs) were primarily observed between positions 1 and 7. They also discovered that most amino acids in the complementary determining region (CDR) interact with the FQs. It is mainly divided into two cases, one is the substitution of atoms on the alkyl chain bound to the antibiotic and the other is the substitution of some functional groups bound to the antibiotic. Using IC50 values, they determined that the mutant m5A7 is commonly used, but it does not enhance affinity for FQ structural analogs. Additionally, for the mutated antibodies in the CDR, the G10E and T21A mutations showed weak effects on antibiotic binding, attributed to their positioning on the periphery of the binding pocket.[21]

Antibodies, as one of the advanced recognition elements, are known for their high specificity and affinity. Currently, the commonly used tests to detect antibiotics as molecular recognition elements include fluorescence immunoassay, lateral flow immunoassay, and enzyme linked immunosorbent assay.

3.2 Fluorescence immunoassay

Fluorescence is one of the most intuitive observational techniques across various fields. In immunoassays, fluorescent labels can be attached to antibodies as tracers, such as fluorophores, fluorescein, nanoparticles, and quantum dots. Fluorescence immunoassays do not require complex sample preprocessing or separation steps, making them efficient and user-friendly.[22] When the target is absent, the fluorescence intensity increases as the fluorophore binds to the antibody. Conversely, in the presence of the target, if the target concentration exceeds that of the fluorophore, the fluorophore preferentially binds to the target, resulting in a decrease in fluorescence intensity. An innovative fluorescence polarization immunoassay was proposed for the detection of ciprofloxacin (CIP) in milk in a study. They first produced a specific monoclonal antibody against CIP and then conjugated the antibody with the carrier protein GInBP, which was isolated from *Escherichia coli*. Finally, they labeled the CPFX-GinBP conjugate with the dye CF647 to obtain a CPFX-GinBP-CF647 fluorescent probe for detection.[23] Building on a different methodology, Huang *et al.* summarized the challenges of detecting norfloxacin (NOR) in milk. They proposed a simple paper-based fluorescence immunoassay by labeling antibodies with quantum dots. The excellent optical properties of quantum dots allow for detection using a fluorescence spectrophotometer. To block other binding sites, they used bovine serum albumin (BSA). The entire system was incubated in the paper for 5 min. As the concentration of NOR increased, the fluorescence intensity decreased. This sensor exhibits a low limit of detection (LOD), high sensitivity, and rapid performance, achieving a LOD of 1 pg mL^{-1} in water and 10 pg mL^{-1} in milk.[24]

3.3 Lateral flow immunoassay (LFIA)

LFIA is a commonly used method in both immunoassays and other types of testing due to its speed, low cost, and high sensitivity, making it widely popular. This convenient detection method has also been prevalent in the point-of-care testing (POCT) field for many years, such as in medical pregnancy tests, as well as in chemical, biological, and environmental testing.[25] Xu *et al.* developed a LFIA capable of simultaneously detecting multiple antibiotics. They used gold nanoparticles (AuNPs) as tracers labeled with antibodies and assembled the components, including a nitrocellulose (NC) membrane, a sample pad, an

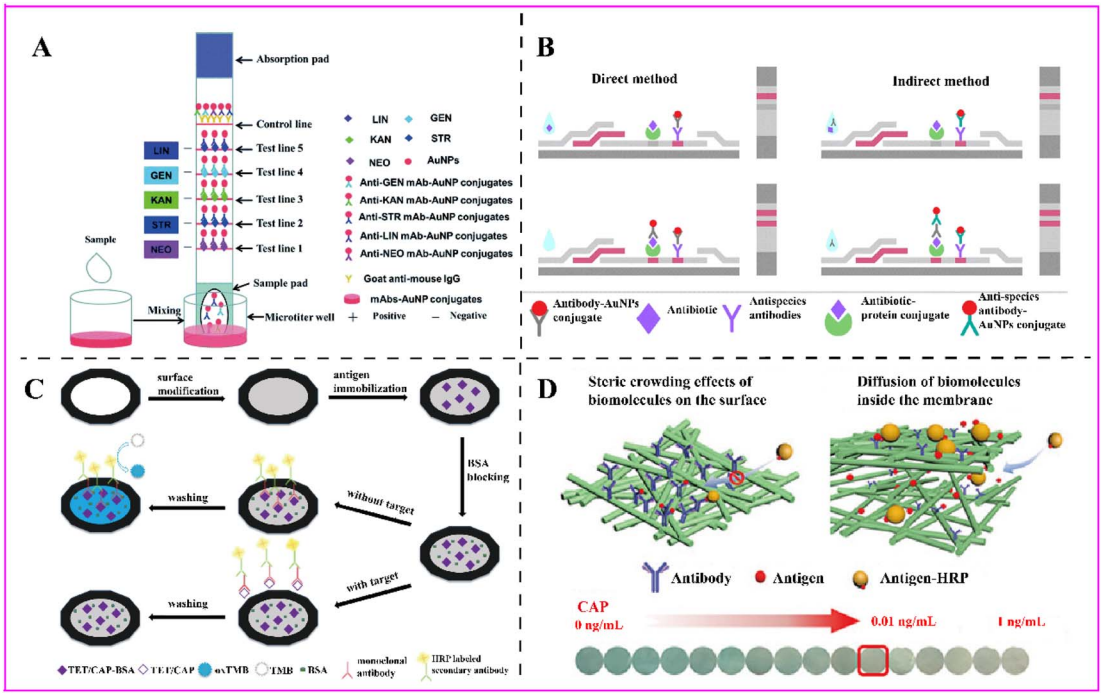

Fig. 2 Antibody-based methods for antibiotic detection. (A) Schematic of the ICA method.[26] (B) Direct and indirect LFIA methods.[27] (C) Schematic of the PAS method.[28] (D) Nanofiber membrane-based ELISA detection method.[29]

absorbent pad, and a PVC support plate (Fig. 2A). Five antibiotics namely lincomycin (LIN), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), and neomycin (NEO) served as antigens, with IgG proteins distributed on the NC membrane to form the test line of the strip. BSA was also used to block the binding sites and prevent degradation. After adding the target into the well, if the target concentration is too high, the T line will disappear, enabling rapid detection.[26] Similarly, research workers used AuNPs to label antibodies and developed two forms of immunoassays for detecting antibiotics in milk, chicken, and honey: direct detection and indirect detection. Direct detection is one of the commonly used methods, while in the indirect method, they combined an unlabeled anti-NEO antibody with an AuNP-labeled secondary antibody specific to the species (Fig. 2B). The entire detection process is completed in 15 min, with a LOD of 10 ng mL^{-1}, and has a wide range of applications.[27]

3.4 Enzyme linked immunosorbent assay (ELISA)

ELISA is a technique that utilizes the specific binding between antigens and antibodies for quantitative analysis and detection. It is further aided by enzyme-substrate reactions that produce a color change. This method is simple to perform, is capable of handling large numbers of samples simultaneously, and has become widely used for detecting antibiotics in food and environmental matrices.[30] Liu *et al.* developed a paper-based antibiotic sensor (PAS) that uses a colorimetric competitive assay and is integrated with a smartphone for detecting TC and chloramphenicol (CAP) in milk and fish samples. The principle is straightforward: antigens are immobilized on hydrophilic micropores and bind to monoclonal antibodies. In the absence of the target, the micropores exhibit a deep blue color. In the presence of the target, the micropores transition to a white color. The antibiotic concentration is quantified by measuring the fluorescence intensity ratio between the detection channels. They also optimized the concentrations of antigens, antibodies, and incubation time to prevent these factors from affecting the LOD. Specificity tests were conducted for TC and CAP analogs, showing good results only for TC and CAP, with no effect on other antibiotics (Fig. 2C). This sensor is characterized by its low cost and rapid detection time.[28] To achieve portable ELISA detection, Sun *et al.* designed a novel nanofiber membrane that reduces steric hindrance and improves antibody diffusion, enabling aptamers to reach an ultra-low LOD. By integrating ELISA with PVA-*co*-PE nanofiber membranes, they increased membrane permeability. Compared to traditional ELISA methods, CAP can be visually detected at 0.01 ng mL^{-1}, and using a smartphone, the LOD reaches 0.005 ng mL^{-1} (Fig. 2D).[29]

Care should be taken during the use of antibodies as molecular recognition elements, especially when immobilized and conjugated on the sensor surface, where some chemical reagents may chemically react with the antibody, causing it to lose its own effect. To maintain the stability of the antibody itself, it is necessary to select a suitable substrate for deposition with the antibody.

4. Aptamer-based detection of antibiotics

Aptamers are selected through an *in vitro* enrichment process known as systematic evolution of ligands by exponential

Table 1 Comparison of aptamer components

Sensing methods	LOD	Detection range	Detection time	Real samples	References
Electrochemical	0.46 nmol L^{-1}	1–10 000 nmol L^{-1}	40 min	Milk	32
Chemiluminescence	143 nmol L^{-1}	0.2–150 μmol L^{-1}	10 min	Water	33
Colorimetric	0.2 pg mL^{-1}	0.0005–30 ng mL^{-1}	30 min	Milk	34
Fluorescence	1.997 ng mL^{-1}	10–50 ng mL^{-1}	60 min	Milk	35
SPR	0.0069 μg kg^{-1}	0.5–500 μg kg^{-1}	70 s	Honey	36
Other	1.89 nmol L^{-1}	0–10 μmol L^{-1}	30 min	Water	37

enrichment (SELEX). This process begins with the synthesis of a library of single-stranded oligonucleotides, which are incubated with the target molecule. Various techniques, including washing and centrifugation, are employed to remove sequences that do not bind to the target.[31] The bound sequences are then amplified using PCR and sequenced to isolate the desired aptamer. The sensors that use aptamers to detect antibiotics are also diverse (Table 1).

4.1 Aptamers–antibiotic recognition mechanism

Compared to antibodies, aptamers offer several advantages, including simple *in vitro* synthesis, the ability to bind to multiple targets, and ease of modification.[38,39] Aptamers are single-stranded DNA or RNA molecules that possess multidimensional conformations. They can fold into structures such as stem-loops, hairpins, and G-quadruplexes, allowing them to tightly bind to their targets through various intermolecular interactions.[40] Strehlitz *et al.* conducted a regional study on aminoglycoside antibiotics and aptamers, highlighting that the amino groups of tobramycin (TOB) exhibit significant hydrogen bonding interactions with the aptamer.[41] Gong *et al.* performed molecular docking studies on the aptamers of tetracycline analogs doxycycline (DOX) and OTC. By analyzing the 3D spatial structures and binding pockets, they identified which nucleobases serve as binding sites between the aptamers and their targets, providing crucial insights for subsequent aptamer modifications and optimization.[42] Guo *et al.* performed molecular docking and secondary structure prediction of the aptamer obtained after screening with penicillin and found that penicillin was tightly connected to the AGGGGAAC portion of aptamer P-11 through hydrogen bonding and π–π stacking interaction forces, thus obtaining the binding pocket of this aptamer with penicillin.[43] Aptamers can serve as molecular recognition elements that combine with signal output to construct various types of biosensors.

4.2 Electrochemical aptamer sensors

Electrochemical aptamer (EAB) sensors have gained widespread application in recent years. They detect and quantify the presence of target molecules by measuring changes in electrochemical signals. When a target molecule binds to an aptamer, the aptamer undergoes a conformational change, which leads to alterations in the electrochemical signal, such as variations in current, potential, or conductivity. By measuring these changes, the sensor can quantitatively analyze the concentration of the target molecule.[44] Common detection methods for electrochemical aptamer sensors include voltammetry, amperometry, potentiometry, and impedance spectroscopy.

4.2.1 Electrochemical aptamer sensors based on voltammetry. Voltammetry is a commonly used electrochemical analysis technique that analyzes samples by measuring the relationship between current and voltage. A reusable EAB sensor was developed for the detection of ampicillin (AMP) in three complex matrices (serum, urine, and saliva). The aptamer for AMP was doubly labeled, with one end modified with a thiol group and the other with methylene blue. The beginning and ending ends of the aptamer are named 5′ and 3′. In the study, the 5′ end of the aptamer was connected to an electrode, while the methylene blue at the 3′ end underwent a conformational change in the presence of the target, facilitating electron transfer. Subsequently, they characterized the constructed sensor using alternating current voltammetry (ACV) and square wave voltammetry (SWV), comparing the LOD for both methods. The LOD for SWV was 30 μmol L^{-1}, while for ACV it was 12 mmol L^{-1}, indicating that SWV is more suitable for detecting AMP in real samples.[45] The use of high-performance nanocomposites allows for better immobilization of aptamers on electrodes. Mahmoud Roushani's group immobilized streptavidin aptamers onto electrodes by means of AuNPs, GQDs-N-S, and AgNPs and monitored the streptavidin binding process to the aptamers by using differential pulse voltammetry (DPV) and cyclic voltammetry (CV). Upon streptomycin binding, the electrochemical signal of the sensor changes, and the signal change is proportional to the streptomycin concentration. The LOD is 0.003 pg mL^{-1} in serum.[46]

4.2.2 Electrochemical aptamer sensors based on impedance spectroscopy. MIPs are artificially synthesized polymer materials that create cavities at the molecular level, designed to have complementary shapes and chemical functionalities to specific target molecules or template molecules.[47] The MIP technique involves introducing the target molecule during the polymer synthesis process, creating specific recognition sites that allow for highly selective identification and separation of that molecule.[48] Azadbakht *et al.* combined MIPs, AuNPs, and TC aptamers for the efficient detection of TC. The process of integrating these three components is relatively straightforward. They first thiolated the aptamers to couple them to the surface of AuNP/glassy carbon electrode (GCE), followed by soaking the electrodes in TC to allow for the binding of free

aptamers. After rinsing the electrodes with water and drying, they used dopamine for the electrochemical polymerization of MIPs. TC was washed out from the MIP cavities using an ethanol-acetic acid solution. This sensor achieves high sensitivity through dual recognition, utilizing the specific affinity between the aptamer and TC, as well as the non-covalent interactions of MIP functional groups with TC. In real milk samples, the sensor demonstrated good recovery rates of 94.9% to 106.2%.[49]

4.2.3 Electrochemical aptamer sensors based on amperometry. Montmorillonite (MMT) is a layered silicate mineral that belongs to the clay mineral family. Its crystal structure consists of two layers of tetrahedral silicon–oxygen (SiO_4) sheets sandwiching a layer of octahedral aluminum–oxygen (ALO_6) sheets, exhibiting significant interlayer cation exchange capacity and expandability.[50] Due to its unique layered structure and high surface area, MMT has garnered significant attention in electrochemical applications, particularly in the modification of electrodes. A research team utilized molecular docking to refine TC aptamers after screening with GO-SELEX. Based on this, they developed an electrochemical sensor using an MMT-modified glassy carbon electrode (GCE) for detecting TC residues in milk. During the sensor construction, MMT was modified with sodium through cation exchange technology to create Fe/Zn-NaMMT. They then coupled multi-walled carbon nanotubes (MWCNTs) to the GCE and with Fe/Zn-NaMMT (Fig. 3A). After drying, the aptamer was added, followed by various concentrations of TC. By measuring the current difference before and after the addition of the target, they determined the LOD to be 0.46 nmol L^{-1}, demonstrating excellent responsiveness.[32]

4.3 Chemiluminescent aptamer sensors

Chemiluminescence (CL) refers to the phenomenon where chemical energy is directly converted into light energy during a chemical reaction, resulting in the emission of photons. This process occurs spontaneously without the need for an external light source, making it a self-sustained light-emitting reaction.[53] Typically, after aptamers bind to their target, the binding event is converted into a luminescent signal through several mechanisms, including direct chemiluminescence, enzyme-catalyzed chemiluminescence, and chemiluminescence of metal complexes.

4.3.1 Direct chemiluminescence. Microspheres are spherical particles with diameters typically ranging from 1 μm to several hundred micrometers. Depending on their applications and materials, microspheres exhibit various functions and properties, such as high surface area, good suspension stability, and customizable surface chemistry.[54] In recent years, they have also garnered significant attention. Chloramphenicol is a broad-spectrum antibiotic commonly used in animal feed, leading to potential residues in animal tissues.[55] Zheng *et al.* developed a CL sensor for the simultaneous detection CAP using carboxylated magnetic microspheres and polystyrene microspheres as separation carriers (Fig. 3B). The binding affinity between the aptamer and CAP is stronger than that with complementary DNA. As the quantities of both targets increase, more aptamers are retained while the complementary strands decrease. Based on this principle, specially designed G bases in the aptamer produce an instantaneous chemiluminescent signal. By plotting the logarithmic values of the target amounts on the *x*-axis and the difference between the high blank values and low signals on the *y*-axis, a standard curve is established, yielding a LOD of 2.48×10^{-8} mol L^{-1} for CAP.[51]

4.3.2 Enzyme-catalyzed chemiluminescence. G-quadruplexes are tetrahedral formations created using sequences rich in guanine. They can exist in three structural conformations: parallel, antiparallel, and hybrid parallel. G-quadruplexes are frequently used in combination with hemoglobin, where they exhibit catalytic functions.[56] A three-dimensional chemiluminescent aptamer sensor has been successfully developed, G4-DNAzyme/S-Apt/β-CD/IL@GOGA, for the highly sensitive and accurate detection of STR. They first synthesized and characterized a graphene-based matrix, β-cyclodextrins and ionic liquid functionalized graphene oxide aerogel (β-CD/IL@GOGA), and then conjugated the STR aptamer with G4-DNAzyme. In the presence of streptomycin, G4-DNAzyme dissociates, catalyzing the reaction between luminol and H_2O_2, which results in a measurable luminescence change. The sensor's stability and reproducibility were validated, showing that it can be reused approximately six times, with a recovery rate of 98.0% to 103.3% in milk samples.[57]

4.3.3 Metal complex chemiluminescence. Some metals can also be used in the construction of sensors. Ma *et al.* developed a chemiluminescent sensor based on a planar luminescent platinum(II) complex and KAN aptamer. Previous studies have demonstrated that platinum(II) complex 1, when inserted into double-stranded DNA (dsDNA), produces intense light emission. Building on this principle, they discovered that in the absence of KAN, the aptamer does not form a double-stranded structure. However, upon the addition of KAN, the aptamer transitions to a double-stranded configuration, resulting in a strong luminescence effect at 545 nm. The LOD for this method is as low as 143 nmol L^{-1}. This approach is simple to operate and suitable for high-throughput detection.[33]

4.4 Colorimetric aptamer sensors

A colorimetric aptamer sensor is a type of sensor based on colorimetric detection, which is a technique that measures the analyte concentration through color changes. This allows the colorimetric aptamer sensor to convert biological recognition events into visible color change signals, making the detection process simple, fast, and free from the need for complex equipment.

LFIA is a type of colorimetric aptamer sensor, widely used due to its rapid detection speed and low cost.[25] Suman *et al.* developed a LFIA test strip using AuNPs and carrier proteins to detect OTC. They first modified the aptamer by molecular docking simulations to retain the core region of the aptamer. Then, OTC was conjugated with the carrier protein and used as the A line, while streptavidin conjugated with complementary DNA was used as the B line, both assembled on a nitrocellulose

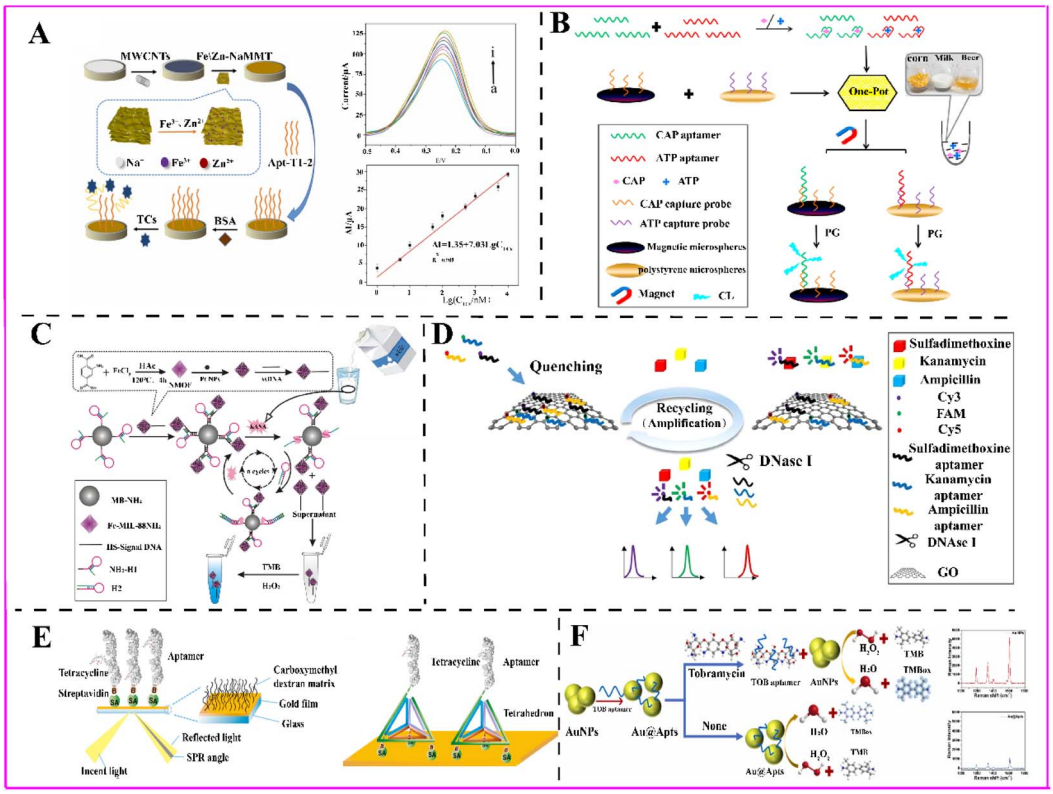

Fig. 3 Aptamer-based methods for antibiotic detection. (A) Schematic of the electrochemical aptamer-based sensor.[32] (B) Schematic diagram of the aptamer sensor for simultaneous detection of CAP.[51] (C) Colorimetric sensor for kanamycin detection based on an NMOF-Pt labeled magnetic L-DNA probe and CHA amplification.[34] (D) Schematic diagram of antibiotic detection using the CESA platform.[35] (E) Schematic of the surface plasmon resonance (SPR) aptamer sensor for detecting TC.[36] (F) Schematic of the aptamer sensor for detecting TOB.[52]

membrane. OTC was added to the LFIA, and color changes in the A and B lines were observed. KAN, AMP, and TC were also tested as specificity controls, resulting in a LOD of 0.245 ± 1.62 ng mL^{-1}, with excellent detection performance in milk samples.[58]

Mimetic enzymes, such as G4-DNAzyme, nanozymes, and metal oxides, share similar catalytic properties and have seen widespread application in recent years.[59] Gan *et al.* developed a colorimetric sensor based on magnetic probes and a catalytic hairpin assembly (CHA)-assisted signal amplification strategy, utilizing a nanozyme as a mimetic enzyme. They employed NMOF-Pt nanoparticles as the enzyme mimic and synthesized probes using L-DNA and magnetic beads (Fig. 3C). Upon the introduction of the target, the DNA probe is captured, triggering the CHA reaction, where hybridized H1 and H2 form a double-stranded structure. The hybridized NMOF-Pt significantly increases and catalyzes tetramethylbenzidine (TMB) in the presence of H_2O_2, producing a blue signal visible to the naked eye. The LOD reached 0.2 pg mL^{-1}, with a detection time of 30 min.[34]

4.5 Fluorescent aptamer sensors

The principle of fluorescence sensors is based on the phenomenon of fluorescence, whereby certain substances, after absorbing light energy, emit a specific wavelength of light in the form of fluorescence. For example, a fluorescent probe is illuminated using an excitation light source, absorbs light energy and jumps to a higher energy state. Similar to colorimetric aptamer sensors, they are favored by researchers for their simplicity, rapid operation, and cost-effectiveness. Fluorescent aptamers can bind with various dyes such as tetrahydrothiophene (ThT), SYBR I, crystal violet, and thiazole orange. Additionally, aptamers can be modified by attaching fluorescent groups such as sulfo-cyanine3 (Cy3), sulfo-cyanine5 (Cy5), and carboxyfluorescein (FAM) to both ends. The introduction of a target molecule induces conformational changes in the aptamer, resulting in either an increase or decrease in fluorescence.

SYBR Green I was first validated in 2004 for its ability to insert into the grooves of double-stranded DNA or bind to G-quadruplexes, emitting a strong fluorescent signal.[60] Taghdisi *et al.* developed a simple fluorescent aptamer sensor using SYBR Green I to detect TC in human serum and milk. Thus, they utilized this property to combine the TC aptamer with SYBR Green I, quantifying the concentration of tetracycline by measuring the fluorescence decrease upon the addition of TC.[61] The LOD was determined to be 63 nmol L^{-1}. The research team developed a multiplex sensing platform called CESA (Fig. 3D). They constructed a fluorescent aptamer sensor for the simultaneous detection of sulfanilamide, KAN and AMP by specifically labeling their aptamers with Cy3, FAM, and Cy5. This

approach utilized the principle of fluorescence quenching using graphene oxide (GO), where the modified aptamers caused fluorescence quenching upon interaction with GO. After adding the three target antibiotics, the aptamers were released from GO, and DNase I was employed to amplify the signal. The LOD for the three antibiotics were determined to be 1.997, 2.664, and 2.337 ng mL^{-1}, respectively.[35]

MOFs are a class of highly ordered porous materials formed by the self-assembly of metal ions or metal clusters with organic ligands through coordination bonds. They hold significant promise in the field of sensing.[62] Gao and Kang *et al.* utilized zirconium-porphyrin MOFs, specifically PCN-222, as a fluorescent quencher. The STR aptamer was conjugated to PCN-222. Under high quantum efficiency (QE) conditions, the fluorescence of the aptamer was sharply quenched. When STR was present, the aptamer bound to it and detached from PCN-222, resulting in fluorescence recovery. The entire detection process took approximately 26 minutes, with a LOD of 0.08 pg mL^{-1}.[63]

4.6 SPR aptamer sensors

SPR is a powerful analytical technique used to study molecular interactions. It enables real-time, label-free detection of these interactions, providing valuable data on binding kinetics and affinity.[64] The principle of SPR is based on the interaction between light and the surface plasmons of a metal film. When polarized light is incident on a metal film typically gold or silver at a specific angle, under certain conditions, the energy of the photons can be transferred to the free electrons on the surface of the film surface plasmons, resulting in resonance. This resonance causes a change in the reflected intensity of the incident light, known as the SPR signal.[65]

The SPR sensor surface determines the sensitivity, considering that some small molecules can be determined using materials instead. Romero *et al.* detected KAN by comparing two kinds of materials, a chemical vapor deposited (CVD) metal substrate and reduced graphene oxide (rGO) applied in the sensor. Characterization showed that CVD immobilized the small molecule more strongly than rGO, and the sensitivity was also reduced by a factor of about seven relative to rGO.[66] Long aptamer sequences sometimes exhibit poor immobilization capabilities with SPR. To address this limitation, Liang *et al.* employed DNA nanostructures; they constructed a tetrahedral DNA framework that enclosed the TC aptamer Apt76 at its apex using four constituent sequences, while biotin was labeled at the base (Fig. 3E). The nanoscale distance of 6 nm mitigated the effects of steric hindrance, allowing for automated detection with the instrument. The recovery rate for the samples ranged from 80.20% to 114.3%.[36]

4.7 Other types of sensors

Other sensors exhibiting optical properties can also be combined with aptamers for antibiotic detection. Surface-enhanced Raman scattering (SERS) is a powerful spectroscopic analysis technique based on the Raman scattering effect. It significantly amplifies molecular vibrational signals through the dramatically enhanced electromagnetic field generated on the surface of nanoscale metals.[67] Zhang *et al.* developed a rapid SERS sensor for detecting TOB with the aid of gold nanoparticles. First, they conjugated a TOB aptamer to the gold nanoparticles. When TOB was added, the aptamer bound to it and detached from the nanoparticles, restoring the peroxidase-like activity of the gold nanoparticles, which catalyzed the colorimetric substrate TMB. SERS was then used to detect the TMB colorimetric signal, with the Raman intensity representing the catalytic activity (Fig. 3F). This method achieved good average recovery rates between 94.4% and 102% in real samples of milk and eggs.[52]

In a different context, a hydrogel is a polymer material with a three-dimensional network structure, capable of absorbing large amounts of water or biological fluids.[68] At the same time, it retains its structure without dissolving, exhibiting functional structural adaptability and excellent resistance to external factors such as pH and temperature changes.[69] The use of hydrogels in combination with aptamers for detection is also quite common. Lin and He *et al.* developed a stable GO–PVA hydrogel for UV detection of trace amounts of TC using polyvinyl alcohol (PVA) and GO. First, the aptamer was conjugated to graphene through π–π stacking interactions, and then PVA was added to form the GO–TC–PVA hydrogel. When TC was introduced, the aptamer specifically bound to it, triggering a strong targeting effect on tetracycline's conjugated double bonds, resulting in a detectable UV absorption peak at 260 nm. They later replaced the tetracycline aptamer with a STR aptamer using the same materials and achieved similarly good results, with a LOD of 1.89 nmol L^{-1}.[37]

Currently, many antibiotic aptamers have not yet been screened using SELEX, and the existing aptamers are predominantly focused on a limited range of typical antibiotics. For future applications of aptamers in antibiotic detection, it is essential to develop aptamers that specifically target various antibiotics. This area requires further exploration and research.

5. Microorganism-based detection of antibiotics

Antibiotics are well-known antimicrobial small molecules that can inhibit the growth of microorganisms and serve as specific targets for them.[70] With scientific advancements, various methods for detecting antibiotics using microorganisms have emerged. Although this approach is not the mainstream method for antibiotic detection, it has gained significant attention due to its accuracy and ability to simultaneously detect multiple samples.

Due to its high sensitivity to antibiotics, *Geobacillus stearothermophilus* is one of the commonly used microorganisms for antibiotic detection.[71] Wang *et al.* developed a microbial inhibition-based method using *Geobacillus stearothermophilus* to detect six major categories of antibiotic residues in food. By supplementing the agar medium with nutrients such as peptone and glucose, they enhanced the bacteria's sensitivity to antibiotics. To differentiate the presence of β-lactam,

sulfonamide, aminoglycoside, tetracycline, macrolide, and quinolone antibiotics, they employed chemical reagents that inhibit specific antibiotics, including β-lactamase, PABA, $MgSO_4$, cysteine, and pH adjustment. The spiked concentrations of each antibiotic varied, with LOD in milk ranging from approximately 1 to 150 μg L^{-1} and in egg samples from 0.5 to 50 μg L^{-1}. Validation of real samples using LC/MS-MS showed higher recovery rates compared to this method, demonstrating its reliability and practicality.[72] Further exploring the topic, *Geobacillus stearothermophilus*-specific spores are produced and found that a combination of 10% skim milk, 4% glycerol, and 12% trehalose resulted in the highest survival rate and lyophilized spore viability. Using SEM, they assessed the impact of nutrients on spore morphology, and FT-IR was employed to analyze the spore structure. With these lyophilized spores, they developed a colorimetric antibiotic detection method based on microbial inhibition (Fig. 4A). The spores were mixed into a medium containing the pH indicator bromocresol purple, a sensitizer, and a hydrogel. In the presence of antibiotics, spore growth is inhibited, and the pH remains unchanged, keeping the medium's color stable. Conversely, if antibiotics are absent, spore metabolism produces acid, causing the medium color to shift from purple to yellow. This colorimetric sensing platform enables the detection of seven representative classes of antibiotics.[73]

Microorganisms can demonstrate measurable growth inhibition even in the presence of low concentrations of antibiotics, resulting in more sensitive test outcomes. Additionally, microbial assays generally require less equipment and are easier to perform compared to other methods. However, one drawback is that microbial culture times can be relatively long. To address this, rapid culture techniques or molecular biology methods can be employed to expedite the acquisition of results.

6. Cell-based detection of antibiotics

Antibiotics may lead to changes in the metabolic activity of cells, and these changes can be monitored by specific biochemical or biophysical methods. On the other hand, certain antibiotics affect the permeability of cell membranes, and changes in cell integrity and function can be used to detect their presence. Therefore, cells can be used as molecular recognition elements for the detection of antibiotics.

6.1 Whole cell sensors

A whole-cell biosensor is a type of biosensor constructed using living cells, designed to detect specific chemicals, environmental changes, or other biological signals.[77] These sensors use intact cells—typically bacteria, yeast, fungi, or mammalian cells—as detection tools, leveraging biological processes such as metabolism, transcription, and translation to recognize target substances and generate measurable response signals.[78] Synthetic biology plays a crucial role in the construction of whole-cell biosensors.[79]

Cheng *et al.* combined smartphone technology, microfluidic chips, and the luminescent gene from *E. coli* to develop

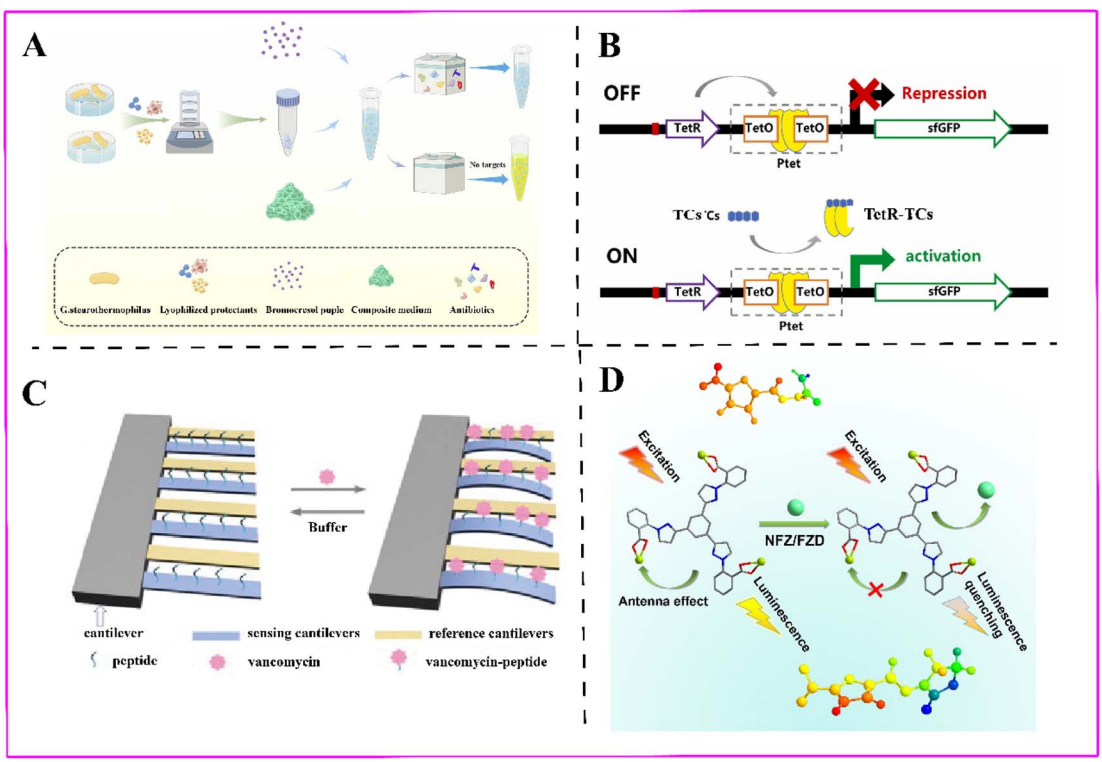

Fig. 4 Microorganism-, cell-, peptide- and MOF-based methods for antibiotic detection. (A) Multiplex platform for detecting multiple antibiotics.[73] (B) Basic principle of a whole cell biosensor for TC detection.[74] (C) Schematic illustration of reversible VAN detection.[75] (D) Mode of Tb-TCPB distinguishing between different types of antibiotics.[76]

a smartphone-based whole-cell microarray sensor, LumiCell-Sense (LCS), for detecting CIP in milk. First, a reporter gene strain with a plasmid containing the recA promoter, known for its high sensitivity to CIP, was cultured overnight in LB medium. The bacteria were then resuspended in 0.4% alginate-LB. In preliminary experiments, cell density was optimized, and colony-forming units were determined by assessing different bacterial response ranges. Additionally, a mobile application, LCS_Logger was developed and integrated with the microfluidic system to process data. LCS_Logger analyzes bioluminescence spectra and correlates them with the time represented by different colors. Whole milk was then used as a real sample, with a LOD of 7.2 ng mL^{-1}.[80] To address the low sensitivity of whole-cell sensors, Chen *et al.* used directed evolution and high-throughput screening to obtain the TC mutant epS2–22 for TC detection (Fig. 4B). During three rounds of screening, mutants epS1–8, epS2–22, and epS3–61 were selected for validation. Results showed that epS1–8 had a high fluorescence background, while epS3–61 had a low response to other tetracyclines, leading to the selection of epS2–22 for further experiments. Directed evolution improved the mutant's affinity and optimized sensor performance. Additionally, molecular docking was used to explore the interaction between epS2–22 and TC. Detection in spiked water samples showed a LOD of less than 10 μg L^{-1}, which was lower than the LOD of the HPLC method.[74]

6.2 Cell-free sensors

Similarly, synthetic biology makes significant contributions to cell-free biosensors. Unlike whole-cell biosensors, cell-free biosensors are not restricted by environmental factors, such as pH, temperature, ionic conditions, and other chemical influences on cells.[81] A sensing platform utilizing the TC heterodimeric transcription factor aTF as a recognition element is developed, combined with two signal amplification methods: strand displacement amplification (SDA) and hybridization chain reaction (HCR). In the presence of the target tetracycline, it competes with aTF for binding, triggering the action of the cut-site nuclease and initiating the SDA reaction. The products released from SDA serve as triggers for the HCR, with H1 and H2 composed of three-quarters and one-quarter of G-quadruplexes, respectively. Based on the principle of strand displacement, when H1 opens, the G-quadruplex self-assembles with ThT, resulting in increased fluorescence. In contrast, when TC is absent, neither the SDA nor the HCRs are triggered, leading to a decrease in fluorescence. To validate the performance of the cell-free biosensor in real samples, tests were conducted using tap water, Yellow River water, Mudan River water, milk, honey, and chicken spiked samples, demonstrating an impressive linear detection range from 20 ng mL^{-1} to 1000 ng mL^{-1}.[82]

By selection of appropriate cell lines to ensure the biological relevance and applicability of the test results, with a gradual increase in the use of antibiotics, antibiotic resistance will gradually increase. Therefore, we need to pay attention to changes in cell resistance to antibiotics and timely adjustment of the detection strategy. What's more, in the field of antibiotic detection, the use of cells as molecular recognition elements is relatively uncommon. However, cells can provide valuable functional information, such as the biological activity or toxicity of antibiotics, which is often challenging to assess with other recognition elements.

7. Peptide-based detection of antibiotics

Peptides are short-chain molecules composed of amino acids linked by peptide bonds, typically consisting of 2 to 50 amino acids. They exhibit a wide range of functions and can serve as signaling molecules, hormones, or alternatives to antibodies[83,84] and can also be used for molecular recognition.[85] Peptides' flexibility and tunability enable them to efficiently bind to specific molecules in the field of biosensing, such as antibiotic detection.

The cantilever sensor is a highly sensitive device based on microelectromechanical systems (MEMS) technology, typically consisting of a microbeam that is freely suspended at one end while the other end is fixed. When antibiotics bind to the recognition elements on the sensor's surface, it induces changes in surface stress or mass on the cantilever, resulting in slight bending or variations in the vibration frequency of the beam. Vancomycin (VAN) is a glycopeptide antibiotic considered a last line of defense against bacterial infections, widely used in clinical treatment and animal husbandry.[86] A research team developed a reversible detection strategy for VAN using a peptide, L-Lys-D-Ala-D-Ala, as the recognition element, which was immobilized on the cantilever. They observed changes in the sensing force after VAN binding. By introducing different concentrations of VAN ranging from 2 to 100 μmol L^{-1}, they measured the cantilever's bending and calculated the LOD to be 0.2 μmol L^{-1}. To evaluate the sensor's applicability, tests were also conducted in serum (Fig. 4C), revealing a LOD of 0.8 μmol L^{-1} in 20% fetal bovine serum, demonstrating its potential for reversible detection of VAN.[75] Sample preparation is a critical issue in food testing. However, if the preparation process is not carried out meticulously, it can easily result in loss of function or impaired sensitivity of the sensing system. In a separate study, Wang and Gu *et al.* developed a multifunctional antifouling peptide, [DOPA3-Pro-Pro-Pro-Pro-Glu-Lys-Asp-Gln-Asp-Lys-Lys-Ala(D)-Ala(D), DOPA3-PPPPEKDQDKKaa]. The PPPPEKDQDK sequence exhibits excellent antifouling properties, while the Kaa peptide serves as the recognition element for VAN. Additionally, they incorporated Au_2O_3 microspheres into the sensor to enhance its conductivity. After immersing the sensor in a VAN solution for 30 min, they achieved a LOD of 0.038 ng mL^{-1}. Subsequently, they spiked milk, honey, and milk powder samples for detection, obtaining recovery rates ranging from 105.3% to 110.8%. These results are comparable to those from commercially available kits, demonstrating good feasibility and precision.[87]

The stability of peptides as molecular recognition elements remains a key challenge, especially in complex biological environments. Enhancing the stability and environmental

resistance of peptides through chemical modification or through peptide binding to other molecules will be a future direction.

8. MOF-based detection of antibiotics

MOFs are distinguished by their high specific surface area, adjustable pore structures, and versatile chemical functionalities. These unique features make them highly applicable in a wide range of fields, including gas storage, separation, catalysis, biosensing, and drug delivery.[88]

8.1 Transition metal-based MOFs

The relatively small size of pure MOF crystals can limit their practical applications and affect their electrochemical or fluorescent performance. To enhance their catalytic activity or improve electrochemical fluorescence signals, they are often combined with metals such as Fe, Zn, or Cu.[89] A 2D CD-MOF has been developed, named LCU-111, based on the luminescence principles of MOFs. They combined this with a mixed matrix membrane (MMM) to create a luminescent quenching sensor for detecting trace amounts of nitrofurazone (NFZ) in water. The 2D structure of the MOFs facilitates easier access to active sites, improving the overall performance of the sensor. The flexibility of the MMM alleviates the rigidity of the MOFs, providing good permeability. As a result, the sensor demonstrates low LOD for NFZ in various environments. Specifically, the LOD is 0.4567 ppm in aqueous solutions, 0.3649 ppm in urban river water, and 0.8071 ppm in HEPES buffer, all achievable within 20 s. Additionally, they created a luminescent paper based on LCU-111 and proposed a fingerprint identification method utilizing its luminescent properties to distinguish personal information in criminal investigations. The fluorescence quenching mechanism was further validated using density functional theory (DFT) techniques.[90]

Aggregation-induced emission (AIE) is commonly used in cellular imaging and bio-detection applications,[91] When fluorescence is weak, molecular aggregation is required to enhance the signal. Sun and Wang *et al.*[92] discovered that gentamicin exhibits a unique fluorescent response with MOFs. Zinc-based MOF, Zn-BTEC, can enhance the AIE of gentamicin. The MOF itself does not exhibit fluorescence; however, upon the addition of trace amounts of gentamicin, the fluorescence intensifies, with a LOD of 28 nmol L^{-1}. This fluorescence enhancement occurs due to the aggregation emission induced by the decomposition or assembly of gentamicin within the MOFs. Gentamicin belongs to the class of tetracycline antibiotics.[93] The study also found that specific fluorescence wavelengths can distinguish between chlortetracycline (CTC) and other TC analogs. Subsequently, MOF materials were immobilized onto portable test strips. When different concentrations of CTC were added and excited at 365 nm, a clear variation in fluorescence was observed with changes in the CTC concentration, indicating the detection range for CTC.[92]

8.2 Rare earth metal-based MOFs

Lanthanides (Ln) are among the most commonly used elements in the rare earth metals group.[94] Ln metal–organic frameworks (LMOFs) are a class of three-dimensional porous materials formed through the coordination of lanthanide metal ions with organic ligands. These materials exhibit excellent thermal and chemical stability, large Stokes shifts, and long luminescence lifetimes.[95] Ln elements can form organic coordination complexes with many antibiotics for detection purposes.[96]

A research team synthesized an ultra-stable Eu-MOF using a hydrothermal method. This metal–organic framework (MOF) features a previously unreported 2D topology (4-c) with two types of ligands: $PIA2^-$ and $HPIA^-$. The luminescent properties of the Eu-MOF were studied, revealing four high-resolution emission peaks at 590, 614, 643, and 696 nmol L^{-1}. It was subsequently employed as a fluorescent probe for detecting quinolone antibiotics in water, such as CIP and ofloxacin (OFLX). To explore the detection of various antibiotics, five classes were tested. It was observed that CIP and OFLX exhibited distinct color changes in fluorescence quenching, transitioning from red to blue and finally to green, which enabled easy identification of CIP and OFLX. The LODs were 0.693 ppb and 0.802 ppb, respectively, demonstrating excellent fluorescent performance and convenient detection capabilities.[97] Zhang and Wu *et al.* developed a dual-core single lanthanide element dual-emission ratiometric fluorescent sensor. The dual-core material, named Tb-TCPB, is a Ln-MOF that detects NFZ and furazolidone (FZD) based on the specific interaction between Tb^{3+} and the ligands (Fig. 4D). First, the stability of Tb-TCPB in typical water environments was investigated using fluorescence spectroscopy, revealing that it maintains excellent stability in the pH range of 5–9. Subsequent tests in spiked tap water demonstrated recovery rates between 97% and 107.3%, confirming the accuracy of the Tb-TCPB sensing platform.[76] Chen *et al.* even further developed a MOF, namely Ce/Zr-UiO 66, by integrating it into a sensor for both CAP detection and pretreatment. The sensor achieved spiked recoveries of 97.32–119.25% in fish, wastewater and urine with rapid detection and accuracy.[98]

The pore structure of MOFs provides a large surface area, which increases the chance of contact with antibiotics and enhances the detection sensitivity. In addition, the stability and reusability of MOFs under different environmental conditions need to be improved to ensure the consistency of detection results.

9. MIP-based detection of antibiotics

MIPs are high-performance materials that are economical and reusable.[99] MIPs demonstrate broad applications in fields such as separation, detection, and catalysis. They are often combined with other detection technologies, streamlining the sample preparation process and reducing the separation steps required by traditional methods. Additionally, MIPs can serve as adsorbents in solid-phase extraction (SPE) in various forms, including magnetic, dispersive, and packed formats.[100]

Ozkan *et al.* developed an alginate@TiO_2/MIP-GCE biosensor for detecting VAN in serum and water using a green material, alginate, along with TiO_2 nanoparticles and MIPs. They first coupled TiO_2 with the target VAN and then incorporated the VAN-coated TiO_2 into alginate, using $CaCl_2$ as a cross-linking agent to prevent biodegradation. Electrochemical measurements were conducted using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS), while optimizing factors such as binding time, drying time, and polymer ratio to ensure optimal sensor performance. Finally, testing on real spiked samples yielded a remarkably low LOD of 2.808 pmol L^{-1}, demonstrating excellent detection capabilities and high biosafety.[101]

Multi-walled carbon nanotubes (MWCNTs) are composed of multiple concentric layers of graphite.[102] Typically, the diameter of each carbon nanotube ranges from a few nanometers to several tens of nanometers. Generally, as the number of carbon atomic layers increases, their strength and toughness also improve, resulting in excellent mechanical properties and conductivity, along with high stability. While the international market price of MWCNTs has been gradually decreasing, their synthesis cost remains relatively high compared to other nanomaterials.[103] Li and Ding *et al.* found that the synergistic effects between bifunctional or multifunctional monomers result in greater adsorption and selectivity compared to monofunctional monomers. They developed a dual-function monomer MIP sensor, MIP-dual/MWCNT-ZIF8/GCE, targeting gatifloxacin (GTX) using *p*-aminobenzoic acid (*p*-ABA) and niacinamide (NA). The incorporation of ZIF-8 (zeolitic imidazolate framework-8) enhances the sensor's conductivity. The sensor preparation process involves three steps. First, the combination of MWCNT-ZIF8 with the GCE; second, the electrochemical polymerization to synthesize the MIPs; and finally, the removal of GTX to create cavities for detection. The sensor's specificity and stability were validated by testing with structural analogs of GTX, such as ciprofloxacin and levofloxacin, revealing that only GTX provided the best results. Additionally, recovery rates for GTX in real samples were determined, showing a recovery range of 96.5% to 105%. This sensor demonstrates significant potential for application in real-world environments.[104]

In the future, it is crucial to investigate more efficient synthesis methods to lower the production costs of MIPs while maintaining consistent quality and performance. Additionally, efforts should focus on reducing cross-reactivity and minimizing detection time.

10. Direct recognition-based detection of antibiotics

Different antibiotics have different molecular properties, such as the structure of the antibiotic itself, charge ratios, solubility, and so on. In recent years, there has been a proliferation of direct assays based on these properties, such as MS, SERS and Fourier transform infrared spectroscopy (FTIR), which analyze antibiotic concentrations using spectra or characteristic peaks. Therefore, this detection method is defined as direct recognition.

MS is a powerful analytical tool that analyzes the composition and structure of samples by measuring the mass-to-charge ratio of ions. The core of mass spectrometry involves ionizing molecules in the sample to create charged ions, which are then separated and detected based on their mass-to-charge ratios.[105] The molecular structure of antibiotics determines their specific mass spectral fragments and peaks. The ion peaks characteristic of antibiotics can help identify the type and concentration of the antibiotic in mass spectrometry.[106] Additionally, MS can be combined with other analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), capillary electrophoresis-mass spectrometry (CE-MS), and tandem mass spectrometry (MS/MS), to enhance its application across various fields.[107]

Currently, MS is widely used for antibiotic detection. Guy and colleagues utilized liquid LC-MS/MS to detect 42 types of antibiotics in honey, validating the procedure at three concentration levels: 10, 20, and 30 μg kg^{-1}, in accordance with international standards. They subsequently detected several classes of antibiotics, including tetracyclines, aminoglycosides, and macrolides. Their analysis also revealed structural analogs of antibiotic families present in the honey samples.[108] Zhang *et al.* developed a porous aromatic framework as a solid-phase adsorbent for food sample preparation. This adsorbent demonstrates excellent adsorption performance for complex matrices. After the sample preparation, LC-MS/MS was employed to detect six types of macrolide antibiotics in chicken, with a concentration of 27.336 μg kg^{-1}.[109] Molina-Díaz *et al.* employed the QuEChERS method for sample preparation, followed by rapid LC-TOFMS to detect sulfonamides and other veterinary drugs in 12 shrimp samples collected from different regions. The overall recovery rate ranged from 58% to 133%, with a regular error of less than 2 ppm, demonstrating high sensitivity.[110]

The SERS technique combines Raman spectroscopy and metal surface enhancement effects for enhancing the Raman scattering signals of molecules.[111] Different functional groups in antibiotics have different effects on the light scattering properties. Antibiotic molecules produce unique Raman signals in specific Raman shift regions, and by analyzing such signals, highly sensitive qualitative and quantitative detection of antibiotics can be achieved.[112]

Clinically, some antibiotics are usually used as antimicrobial drugs for treatment, so there are strict requirements on the amount of antibiotics to be used. Li *et al.* used two kinds of bromide ions and calcium ions to improve the state of substrate silver nanoparticles, which can exclude the interference of some unwanted signals, be used to detect different classes of antibiotics in serum, and also be used to determine different antibiotic molecules according to the different peak intensities derived, and the limit of detection for moxifloxacin can reach 0.05 μg mL^{-1}, which is fully in line with the detection of antibiotics at low levels and is worthy of promotion.[113]

FTIR is a spectroscopic technique that is used to analyze molecules based on their absorption properties of infrared light. It is widely used in the fields of chemistry, materials, biology, pharmaceuticals and environmental sciences to study processes such as molecular structure and chemical composition determination, chemical reactions and environmental detection.[114] Manas Kanti Deb *et al.* revealed that fluoroquinolone antibiotics in food can be detected by using FTIR, which requires pre-treatment for most experiments, whereas this method can be used for direct detection without preconcentration. The analyzed peaks in the FTIR spectrum were found to be CIP and NOR at 1627 and 1026 cm respectively, which allowed the detection of antibiotics at the nanogram level.[115]

However, the field currently requires specialized personnel and sophisticated equipment, while the cost is relatively high and there are relatively few portable MS instruments. Similarly, SERS requires higher-cost instrumentation and materials, thus limiting the POCT.

11. Environmental implications

As highlighted in the introduction of our article, the most critical component of a biosensor is its recognition element. Among the molecular recognition elements discussed in this paper, antibodies, aptamers, microorganisms, cells, and peptides fall under the category of biomolecules. These elements pose minimal environmental hazards, are recyclable, and generate low levels of pollution. For MOFs and MIPs, their production process itself involves some metal compounds and so on, which are more difficult to degrade and will cause more pollution to the environment.

Biosensors have a variety of signal output methods, electrochemical, fluorescence and colorimetric. In the process of making and producing biosensors, some metal materials and chemical reagents are inevitably used, which may cause environmental pollution. In electrochemical sensors, metals such as lead, mercury and cadmium are used as working devices such as electrodes and catalysts. In addition, toxic reagents (methylene chloride, chloroform, *etc.*) and cross-linking agents (glutaraldehyde) are toxic to the human body and may trigger allergic reactions.

Moving forward, the development of biosensors is increasingly focused on sustainability, emphasizing the importance of creating eco-friendly, reusable, and recyclable sensors.

12. Summary and future perspectives

While antibiotics have brought numerous conveniences to our lives and production, they have also given rise to significant issues, such as bacterial resistance and the residual presence of antibiotics in food, pharmaceuticals, and the environment. With advancements in science and technology, methods for detecting antibiotics have proliferated. This review summarizes the research progress of seven types of molecular recognition elements used for antibiotic detection: antibodies, aptamers, microorganisms, cells, peptides, MOFs, MIPs and direct recognition. It provides a brief overview of their recognition mechanisms for antibiotics and the biosensors constructed based on various molecular recognition elements (Table 2).

Recognition elements are a crucial component of biosensors. For historical recognition elements, their limitations are summarized and strategies for future use are proposed. (1) Initially, antibiotic detection primarily relied on antimicrobial susceptibility tests, with the agar diffusion method being the most well-known. This method evaluates the antimicrobial effects of antibiotics based on bacterial growth in culture media, and while it is still in use today, it has significant limitations, particularly its inability to accurately quantify antibiotic concentrations. Future applications of microorganisms as recognition elements for detection could benefit from genetic engineering to enhance their recognition efficiency. Additionally, microorganisms have stringent growth requirements, necessitating careful monitoring of their growth and response to antibiotics. (2) Next, antibody-based detection methods, such as the widely recognized ELISA technique or immunoassays, have emerged. However, these methods face challenges, including high synthesis costs, lengthy production times, and

Table 2 Comparison of 7 molecular recognition elements with direct recognition

Molecular recognition elements	Advantages	Disadvantages
Antibody	High affinity and specificity	High production costs, poor stability, and immunogenicity
Aptamer	Synthesizable, low cost and stable	Optimized affinity and lower thermal stability
Microorganism	Versatile and easy to operate	Demanding environmental conditions requiring handling and incubation
Cell	Strong natural recognition properties, allowing for complex responses	Complex experimental conditions requiring long incubation times
Peptide	Flexible design and low cost	Poor affinity and specificity
MOF	High specific surface area, good adjustability, and good stability	Complex preparation process and high synthesis cost
MIP	Lower preparation costs and high chemical stability	Poor selectivity
Direct recognition	High precision and accurate result	High cost

stability issues influenced by environmental factors. To improve subsequent detection, antibodies can be combined with functional groups such as fluorescence or utilized as bispecific antibodies for detecting multiple targets. Moreover, optimizing the structure of antibodies can enhance their stability. (3) In future detection scenarios, cells are likely to follow a similar trend of modification to antibodies and microorganisms, with the potential integration of automation, high-throughput technologies, and multiplex detection platforms for diverse antibiotic assays.

Compared to the three traditional recognition elements, aptamers, MIPs, and MOFs are popular new recognition elements of the 21st century. Based on the above three molecular recognition elements to propose some ideas of what might be realized in the future, grouping MIPs and MOFs together as they have broadly similar recognition properties. (1) Aptamers function similarly to antibodies, but they have gained attention for their low cost and high specificity due to their accessibility. The functionality of aptamers depends not only on their sequence but also closely relates to their spatial structure. High-resolution structural data of aptamer–antibiotic complexes can be obtained using techniques such as X-ray crystallography, nuclear magnetic resonance (NMR), and cryo-electron microscopy. These data will provide a theoretical basis for the structural optimization and functional tailoring of aptamers. Riboswitches and aptamers belong to the category of functional nucleic acids. Riboswitches are specific RNA sequences that share similarities with aptamers. It remains to be investigated whether riboswitches can undergo conformational changes upon binding to specific molecules, such as antibiotics.[116] This allows for the regulation of gene expression. If designed appropriately, riboswitches can achieve specific recognition of particular antibiotics. Ribozymes are RNA molecules with catalytic activity that can alter their conformation upon binding to target molecules, leading to catalytic reactions.[117] In the future, this characteristic can be leveraged to design platforms for antibiotic detection. (2) MIPs and MOFs share similar properties; however, some metal materials may exhibit low biocompatibility and sometimes have limited detection sensitivity. To address this, they are often combined with nanomaterials to enhance signal amplification. The small size and tunability of nanomaterials make them suitable for *in situ* detection, eliminating the need for complex pretreatment steps. By utilizing the unique catalytic activity and optoelectronic properties of nanomaterials, combined with various molecular recognition elements, it is possible to directly and rapidly detect antibiotics in complex matrix samples. Additionally, the regenerative capabilities of MIPs and MOFs are noteworthy, although their synthesis can be costly. (3) In the future, we believe that we can expand the application of direct recognition modes by integrating various technologies, such as MS and SERS, and at the same time improve the efficiency of preprocessing and minimize the impact of the experimental environment for these sophisticated instruments. What's more, portable testing equipment is also one of the requirements for future development to meet the rapid testing needs faster. Therefore, future antibiotic detection efforts should focus on improving detection efficiency, enabling multiplex detection, ensuring environmental sustainability, and establishing standardized testing procedures. By integrating advanced detection technologies with innovative molecular recognition strategies, we hope to effectively address the challenges of antibiotic monitoring and management.

Data availability

This review did not incorporate primary research findings, software or code, nor did it generate or analyze new data.

Conflicts of interest

There are no conflicts to declare.

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