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Recent developments in fluorescent aptasensors for the detection of antibiotics

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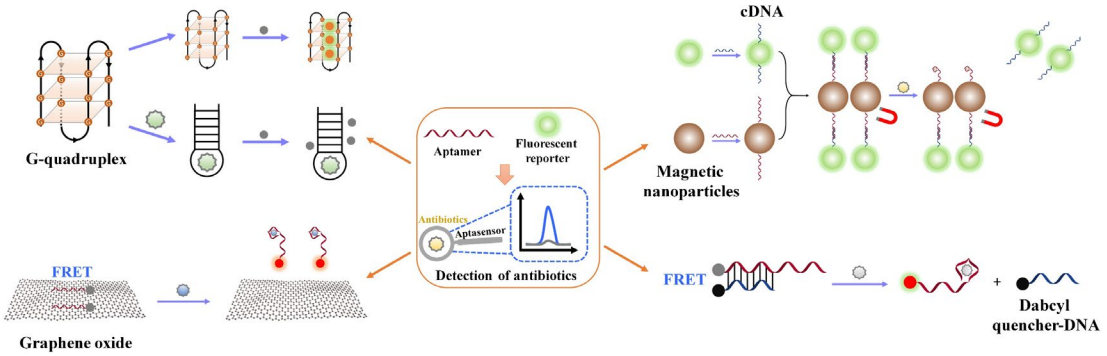
Abstract

Antibiotic abuse is considered as a serious problem affecting to human health, demanding a great attention to explore robust, accurate, real-time, on-site and sensitive methods for rapid evaluation (detection and quantification) in food and biological samples such as serum. To address this challenging problem, biosensors have been developed as a valuable and sensitive tool to detect and quantify the amount of antibiotics. Among various kinds of biosensors, recently aptamer-based biosensors (aptasensors) based on fluorescent strategy have evolved as an excellent candidate for rapid evaluation of antibiotics, owing to their superior selectivity, specificity, and sensitivity. This review encompasses an overview of various kinds of recently-developed fluorescent aptasensors for antibiotic detection and gives an idea of important sensing mechanisms associated with the developed aptasensors. The current focused review aims further to encourage and inspire the targeted readers to develop new approaches for fabricating more practical and mature fluorescent aptasensors for antibiotic detection in the future.

Keywords

Antibiotics, Fluorescent, Aptasensors, Aptamers, Biosensors, Detection of Antibiotics

Graphic abstract



Introduction

Antibiotics, which possess the advantages of strong antibacterial activity and low cost, have been widely used for human medical treatment and veterinary. However, the abuse of antibiotics could result in their accumulation in human body or foods and thus lead to public health issues, for instance, causing ototoxicity, nephrotoxicity, allergic reactions and bacterial resistance [1]. Therefore, it is crucial to develop sensitive, simple, facile, selective and rapid analytical methods for monitoring antibiotic level in foods and human body, which may further improve food safety and facilitate proper drug administration.

Common analytical methods include high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC/MS), capillary electrophoresis (CE), gas chromatography (GC), etc., which are capable of simultaneous detection and quantification of antibiotics. However, these methods usually require expensive/large equipment, professional operators, time-consuming and laborious procedures, limiting their on-site detection and real-time monitoring potential [2]. Biosensors seem to be a good candidate meeting the requirements of antibiotic monitoring, which offer the benefits like high sensitivity, good selectivity for rapid analysis of wide variety of samples [3, 4]. Biosensors consist mainly of a biorecognition element and a signal transducer/reporter, which in fact have been successfully applied in onsite biomedical diagnosis [5]. Enzyme-linked immunosorbent assay (ELISA) is one of the most sensitive and powerful immunological biosensing approaches, which has already proven its great value in the market of diagnosis. However, this antibody-based method suffers from the issues such as the use of animal for antibody production, time-consuming and expensive mode of antibody generation, short shelf life and the potential cross-reactivity problem of antibodies. Aptamers, an alternative of antibody may overcome these drawbacks [6].

Aptamers are typically oligonucleotides (single-stranded DNA or RNA) generated *in vivo* via Systematic Evolution of Ligands by EXponential enrichment (SELEX), which can bind to a wide range of targets such as ions, small molecules, drugs, proteins and whole cells, with high specificity and affinity [7, 8]. As biorecognition elements for biosensors, aptamers possess the advantages of low molecular weight, facile modification, adaptive binding, simplicity of synthesis and good stability [9-11]. Therefore, various aptamer-based biosensors (aptasensors) have been developed for the analysis of antibiotics during the last decade, among which fluorescent strategy is one of the most popular approaches and will be discussed in this review [12, 13]. Fluorescence is a process of

photon emission by a material (i.e., fluorophore) being illuminated with excitation light ($\lambda_{em}>\lambda_{ex}$, Stokes shift), which can be affected by surrounding environment [14]. Herein, we summarize and highlight the recent progress of fluorescent aptasensors for the detection of antibiotics and aim to give the readers an overview and insight of this field. Additionally, we also expect more practical and mature aptasensors to be explored in near future to improve human health.

Fluorescent aptasensors for the detection of antibiotics

Fluorescent analysis is a widely used optical approach in the construction of biosensors which is rapid, sensitive, easy to manipulate and cost-effective. On the other hand, aptamers are known to undergo conformational change upon adaptive binding with its targets, which supply a basis to induce fluorescent signaling/quenching [11, 12, 15-17]. By utilizing the nucleic acid nature and adaptive binding property of aptamers, numerous fluorescent aptasensors have been designed and developed for detection of antibiotics and already reported with various mechanisms, including G-quadruplex/probe complex, competition between complementary DNAs (cDNAs) and analyte, energy transfer, etc [18-22].

G-quadruplex-based Label-free sensing

Aptamers are known to undergo conformational change upon binding to their targets (also known as "adaptive binding"), where one of the results is the disruption of G-quadruplex, a higher-order nucleic acid structure of guanine-rich sequences [20]. On the other hand, some dyes display significant fluorescence variation in the presence of G-quadruplex [23]. By incorporating the aforementioned attractive characteristics of aptamers and the dyes, G-quadruplex-based label-free aptasensors for antibiotics have been designed and developed to remove complicated, time-consuming and expensive covalent-labeling procedures, and to avoid modification-induced decrease in binding affinities of aptamers. Dyes capable of interacting with G-quadruplex, such as thiazole orange (TO), thioflavin T (ThT), SYBR Green I and Nmethylmesoporphyrin IX (NMM), have been explored for the label-free approaches for fluorescent aptasensing, generally based on the deformation of G-quadruplex/dye complex, *via* adaptive binding of aptamers with their specific target molecules [13, 20, 24, 25].

Figure 1 demonstrates a G-quadruplex-based label-free aptasensing of antibiotics. Without the formed G-quadruplex, the dye displays no or low fluorescence. When the dye interacts with G-quadruplex, a significant fluorescence enhancement is observed. In the presence of aptamer target (e.g. antibiotic), aptamer undergoes adaptive binding and results in disruption of G-quadruplex, which further inhibits the formation of G-quadruplex/dye complex resulting a decrease in overall fluorescence intensity. Thus, a quantitative detection method (generally "turn-off" mode) for antibiotics can be established [13, 20]. By employing this strategy, detection of tetracycline has been achieved and compared with HPLC analysis in two individual studies using different fluorescent reporters SYBR Green I and TO respectively, indicating the approach may find its application be applied universally [13, 20].

Similarly, Zhu et al. designed an aptamer beacon with two tandem kanamycin aptamer sequence and a G-quadruplex structure. A fluorescence decrease of ThT was observed upon kanamycin binding and a limit of detection (LOD) of 0.37 nM was obtained [26]. Additionally, their group reported another strategy by designing two probes complementary to kanamycin aptamer. The probes contained the extract sequences, which formed G-quadruplex structure only when the probes

complemented with kanamycin aptamer. The presence of kanamycin folded the kanamycin aptamer and prevented the G-quadruplex formation, resulting in decrease of ThT fluorescence [27]. Interestingly, rather than *via* destroying the formed G-quadruplex, Xing et al. reported a "turn-off" aptasensor by utilizing fluorescent intercalator displacement method with kanamycin. It is claimed that the antibiotic kanamycin displaced the TO (high fluorescent molecule in G-quadruplex) from the G-quadruplex of aptamer, evidenced from nondenaturing PAGE image and CD spectra [23]. To improve and maximize a binding reaction, an aptasensor based on catalytic hairpin assembly reaction and displacement of G-quadruplex was developed. The design employed the target tetracycline drugs for repeated cycles of aptamer binding, and thus reducing fluorescence of the probe. In addition, the sensor was able to simultaneously detect four tetracycline drugs due to the multi-binding capacity of the selected aptamers [25].

Other than directly connecting G-quadruplex to aptamers or exploring the intrinsic G-quadruplex within aptamers, aptamer can also be developed as a "cap" for a guanine-rich sequence. For instance, in the absence of target, a designed tetracycline aptamer can cap a guanine-rich sequence and prevent its formation of G-quadruplex for ThT signaling. In the presence of tetracycline, aptamer binding leads to the release of uncapped guanine-rich sequence, making it available for the construction of triple-helix molecular switch (THMS) and G-quadruplex/ThT complex, and finally results in "turn-on" sensing mechanism [24].

As demonstrated above, by using G-quadruplex/fluorescent dye complex as a signal transduction probe (STP), label-free aptasensors have been designed and developed, which avoids complicated and laborious chemical modification, demonstrating the strategies for simple and facile construction of fluorescent aptasensors.

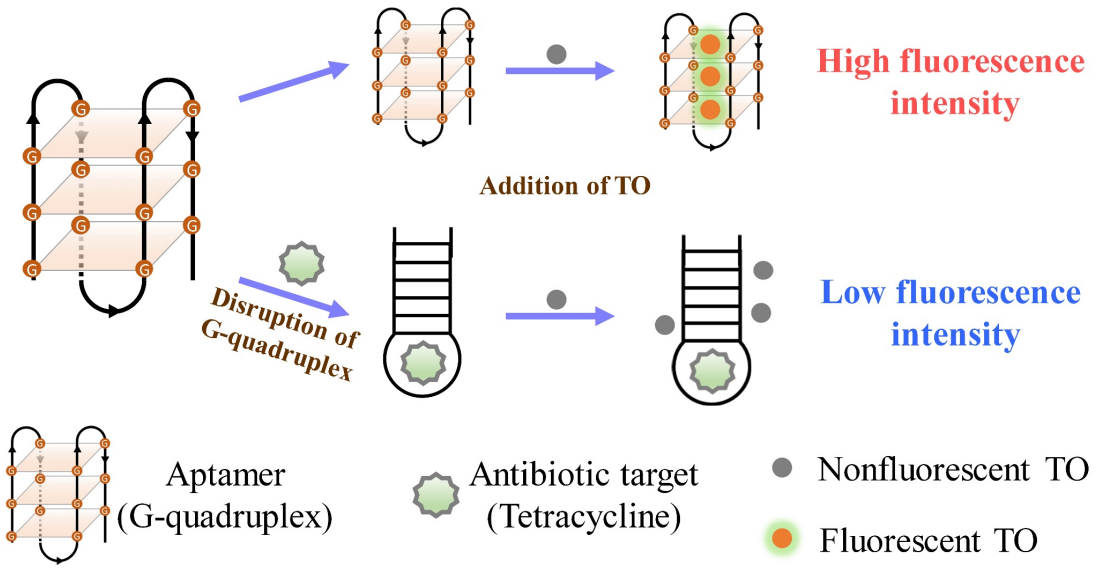


Figure 1. An illustration of the G-quadruplex-based label-free sensing of antibiotics (tetracycline). Figure reproduced from reference [20].

Detection via liberation of cDNAs induced by analyte binding

Considering the natural property of nucleic acid aptamer, fluorescent aptasensors for antibiotic detection have been explored based on the intrinsic base-paring between aptamers and their complementary sequences. In this strategy, which relies on the competition between cDNA and

aptamer target, the aptamer/target binding unwinds or inhibits the base-paring of aptamer/cDNA. Thus, a sensing mechanism with fluorescent change is designed based on the separation of base pairs (demonstrated in **Figure 2**). Based on this concept, a few upconversion nanoparticles (UCNPs) linked with aptamer cDNA have been used as fluorescent reporters. When the cDNA-grafted UCNPs are concentrated with aptamer-immobilized magnetic nanoparticles (magNPs) via paring interaction, a strong fluorescent signal can be observed. The presence of antibiotic analyte unwinds the duplex structures and releases the cDNA-UCNPs, resulting in decrease of fluorescent intensity [18, 28]. To reduce the non-specific absorption of aptamers on magnetic beads, gold nanoparticles (AuNPs) may be used [29]. In another study, Liu et al. employed different probe-labelled cDNAs for simultaneous analysis of oxytetracycline and kanamycin, where the presence of analytes (oxytetracycline and kanamycin) inhibited the formation of probe-cDNA/aptamer complex, and thus reduced the fluorescence signal [30].

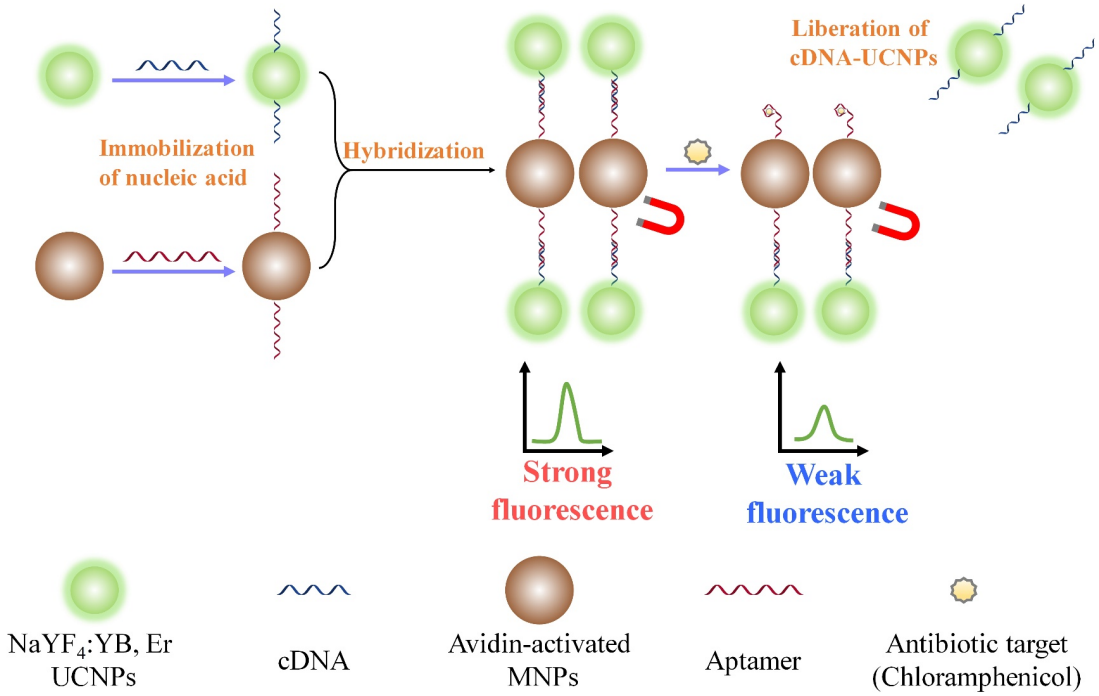


Figure 2. A representative example of antibiotic (chloramphenicol) detection via base pair unwinding induced by aptamer binding. Figure reproduced from reference [18].

Apart from the above principle of accumulating the signal of UCNPs, aggregation of quantum dots (QDs) for fluorescent quenching can also be utilized. For example, in a report from Wang et al., double-stranded DNA antibodies (dsDNA Abs) were immobilized on CdSe QDs, and the fluorescence of CdSe QDs was quenched when dsDNA was added as a bridge. The aptamer binding to the added analyte chloromycetin unwinded the dsDNA and disabled the antibody binding, resulting in separation of QDs and recovery of fluorescence [31].

Furthermore, label-free strategies are also employed usually using nucleic acid stains, for instance, SYBR Gold. It was reported that streptomycin/aptamer binding could lead to the release of cDNA and the protection against Exo III function, facilitating the fluorescent signaling of SYBR Gold (a dye exhibits strong fluorescence enhancement upon binding to nucleic acids) [12]. In another design, Zhang et al. applied microchip electrophoresis (MCE) and hybridization chain reaction (HCR) to

generate duplex DNA chains with different lengths by consuming the nucleic acid H1 and H2, in the presence of kanamycin. Owing to the reduced H1 and H2 triggered by released cDNA from aptamer/kanamycin binding, fluorescent signal of SYBR Gold decreased [32]. Similarly, the cDNA released from antibiotic/aptamer binding can also be analyzed by the application of commonly used Nb.BbvCl nicking endonuclease and Taqman probe [29].

Energy transfer-based detection

The conformational change characteristics of aptamer facilitates the molecular distance variation, which makes energy transfer-based detection possible. One of the frontier methods in energy-based detection is Förster (or fluorescence) resonance energy transfer (FRET)[33-37]. FRET follows dipole-dipole coupling mechanism, the efficiency of which is greatly dependent on critical molecular distance or band gap [38]. The choice of fluorescent molecule including donor, acceptor and quality of the quencher can greatly affect the performance of FRET in biosensing [39]. Aptamers formed of oligonucleotides have been reported to bring the donor and acceptor hairpin moieties (i.e. carboxyfluorescein (FAM), cyanine 3 (Cy3), Green fluorescent protein (GFP)) in close intramolecular armstrong proximity for efficient electron transport and FRET [40].

In line with this, Zhang et al. reported the use of low oxidation degree graphene oxide (GO) as fluorescent quenching substrate, which was able to adsorb aptamer and an aggregation-induced emission molecule (AIE) for label-free fluorescent sensing. The recognition element chloramphenicol aptamer and 9,10-distyrylanthracene (DSA) derivative with short alkyl chains (9,10-bis{4-[2-(*N*,*N*,*N*-trimethylammonium)-ethoxy]styrene}anthracene dibromide, $DSAC_2N$, an AIE) got adsorbed onto GO and quenched. In presence of target chloramphenicol, it formed a complex with aptamer and limited the adsorption of $DSAC_2N$ on to the GO, as a result, liberating $DSAC_2N$. The $DSAC_2N$ then aggregated around the aptamer/target complex, which could turn 'on' the fluorescence. Additional advantages of such system are strong specificity, high sensitivity, cost effective and reusable [41]. A Long-chain aptamer with labelled partially cDNA was further designed to advance the conventional aptamers. Yuan et al. designed partially hybridized oxytetracycline long-chain aptamer paired with FAM fluorophore labelled short-chain ssDNA on graphene for FRET-based sensing of oxytetracycline. In the presence of target antibiotic, the formation of aptamer/target complex left the FAM-labelled cDNA. The cDNA was then paired with its own complementary ssDNA and detached from the graphene sheet, resulting in fluorescence. Further, this design without directly labelling on aptamers avoids the negative effects of the intrinsically existing secondary structure of aptamer [42]. These techniques were superior in terms of analytical specificity and cost effectiveness, but compared to other conventional techniques, it did not offer rapid detection. A novel system by Wang et al. composing of carbon dots and layered MoS_2 promised to overcome this obstacle. The bulk MoS_2 were exfoliated in liquid phase into single or few layer films, and the carbon dots were labeled with DNA probes. The system goes into "on-off-on" 3-stage transition due to assemble of carbon dot labeled aptamer on MoS_2 surface, resulting in fluorescent quenching. In the presence of target kanamycin, strong binding between aptamer and target resulted in free carbon dots, exhibiting high specific fluorescence recovery and achieving desired detection time [43]. Furturemore, an advanced antibiotic sensing system capable of multiple analyte detection is on-demanded. Youn et al. reported a GO based-method for simultaneous measurement of three different types of antibiotics sulfadimethoxine, kanamycin, and ampicillin respectively. The fluorophore-labeled aptamer displayed fluorescent quenching when adsorption on GO was due to FRET. The presence of antibiotic analyte detached the aptamer from GO, and

induced fluorescence recovery of the fluorophore (**Figure 3A**). By incorporating three individual aptamers and three fluorophores, a multiple detection was achieved. Additionally, the system also applied to enzyme assisted cyclic enzymatic signal amplification [22]. Aptamer structure switching-based sensing represents a crucial milestone in FRET aptasensing. Ma et al. developed structure-switching moieties by hybridizing FAM fluorophore-labeled kanamycin-binding aptamer (FDNA) with short oligonucleotide dabcyl quencher DNA (QDNA), forming a double helix structure (diameter = 2 nm) with fluorescent quenching of FAM. In the presence of the target, it formed FDNA/target complex and got separated the fluorophore and quencher, significantly enhancing the fluorescence intensity (demonstrated in **Figure 3B**) [21].

Collectively, these findings suggest the FRET-based assay system are easier to operate and faster than conventional systems, due to utilization of advanced 2D materials and newer aptamer design concept. We suppose that the development of such smart bio-sensing systems is expected to lay the foundation to quantitatively analyze antibiotics prevalent in various systems that could have considerable impact on human health.

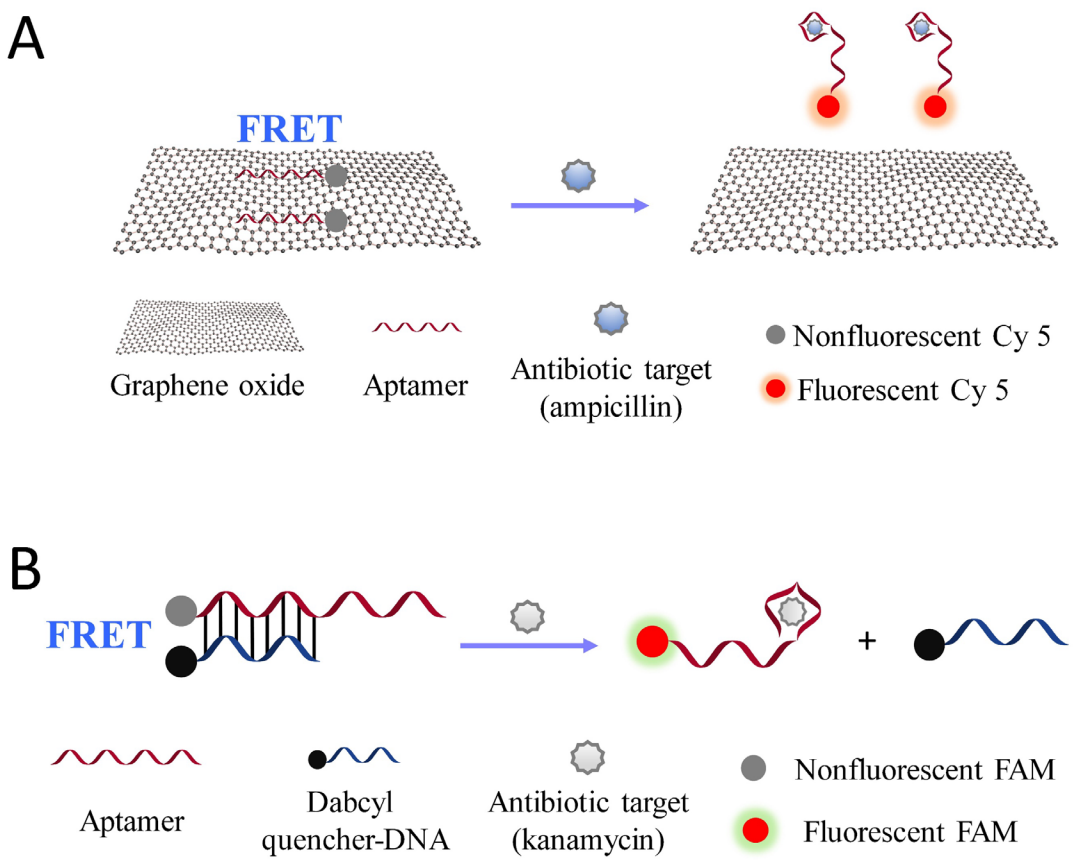


Figure 3. A: A Schematic illustration of FRET-based detection of antibiotics (ampicillin) in graphene oxide/Cy5-Aptamers complex. B: Structure-switching fluorescent aptasensor for antibiotic (kanamycin) detection based on based-pairing and FRET. Figure reproduced from references [21, 22]

Other mechanisms

Additionally, other mechanisms introduced some novel bioengineering techniques, which have been

applied to develop fluorescent aptasensors for antibiotic detection. Chen et al. [44] reported a ratiometric assay based on microchip electrophoresis using a novel R-shape DNA probe to detect kanamycin. The R-shape DNA probe consisted of one single-stranded DNA capturing kanamycin and one hairpin DNA, which would separate when incubated with kanamycin and applied through electrophoresis. The ratio of fluorescent signals of the separated DNA could be calculated to quantitate kanamycin, and the ratiometric detection was good to remove matrix interferences. The application of microchip electrophoresis is beneficial to reduce sample consumption and improve automation efficiency. In another strategy, a label-free fluorescent aptasensor was designed and developed by Dehghani et al. [19] using mesoporous silica nanoparticles to detect kanamycin in a simple and rapid process. The porous particles were filled with Rhodamine B and capped with kanamycin aptamer. The aptamer sequence was separated when kanamycin was presented to uncover the pore and release Rhodamine B (RhB) as fluorescent reporter. Additionally, a highly selective fluorescent aptasensor has been fabricated by Geng Y et al. [45] based on molecular imprinting polymerization technique. In this system, CdSe quantum dots were applied and supported, and aptamer and methacrylic acid acted as functional monomers. The dual recognition of the aptamer and imprinted cavities facilitated highly specific determination of kanamycin. To accomplish high sensitivity, exonuclease-assisted target recycling was commonly incorporated into detection platform for signal amplification [44, 46-48]. In addition, chain reaction such as Polymerase Chain Reaction (PCR) and HCR were applied for signal amplification to have a good sensitivity. To facilitate chain reaction, magnetic beads or bars as aptamer carriers were frequently used to separate capture probes and complementary probes [49-51]. These methods were highly sensitive but required multiple steps.

Strategies for way forward

Overall, fluorescent method has demonstrated its wide application in medical diagnosis and food safety monitoring because of its high sensitivity, flexibility and selectivity. Development of novel fluorescent aptasensors for accurate detection of antibiotics is an emerging and unmeet clinical and environmental need. A plenty of fluorescent aptasensors have already been developed employing various mechanisms discussed above. Unfortunately, most of the developments are still not fully satisfactory to accurately detect and quantitate the antibiotics in food, tissue and environmental samples because the fluorescent signal can easily be interfered by variety of extrinsic and intrinsic factors, which are very difficult to control. There are still many challenges need to overcome and some of the key areas should carefully be considered over next few years to progress the field forward to realize more mature fluorescent aptasensors. For examples, aptasensors via G-quadruplex-based label-free sensing mechanism have some limitations such as interference of other competing ligands. Therefore, ligand competition studies are needed to confirm the selectivity of the ligand of specific interest. This will further address the false positive and false negative results produced by other competing ligands. In addition, few ligands are reported to effectively penetrate the cells and reach their desired target. Therefore, permeability, affinity, and selectivity are critical factors need to be considered and further improved to design and develop advance novel fluorescence G-Quadruplex probes to accurately detect the presence of the antibiotics of interest. Although the aptasensors based on liberation of cDNAs smartly utilize the natural nucleic acid property of aptamers, they face some problems. For instance, when applying this kind of detection in human samples, complementary or partially complementary sequence from samples may induce

the interference. In addition, the “competition affinity” between analyte and cDNA may also affect the final detection outcome. To improve the performance, careful design of cDNA with high binding affinity is suggested to avoid the interference from common nucleic acid. Despite of the significant developments of FRET-based aptasensors, high-fluorescent resolution, improved specificity of the reaction, and the affordability of FRET reagents are still desired in this field, making the strategy for enhancing the ratio of signal to noise as a major challenge. Future work should address on the fabrication of new FRET systems such as multi-FRET aptasensors. In addition, more fluorophores in multi-step FRET system could be used for the detection of the antibiotics of large molecular structure.

Conclusion

Uncontrolled use of antibiotics may induce health issues such as nephrotoxicity and bacterial resistance. In parallel, low-cost, sensitive, rapid, on-site and accurate detection of antibiotics is highly desired. This review summarizes the recent advances in fluorescent aptasensors for the detection of antibiotics, as listed in **Table 1**. Fluorescent biosensing is one of the most widely used approaches due to their abovementioned advantages. In particular, fluorescent aptasensors have been developed for a wide range of analysis. Aptamers not only have common benefits over antibody in stability, generation and modification, but also possess some unique properties of adaptive binding and the nucleic acid nature, which facilitates the design of fluorescent aptasensors, for instance, by combination with the fluorescent quantum principle. The reported fluorescent aptasensors for antibiotic detection have been showing high sensitivity, good selectivity, ease in fabrication, rapid assay, and availability for real sample test. However, it is worth noting that there are rare products in the market. Fluorescence analysis is a sensitive optical approach, but it usually suffers problems such as background fluorescence or auto-fluorescence. Although aptamers have exhibited their advantages over antibodies and can theoretically be selected against any targets, the number of reported aptamers is still limited to date. This bottleneck may prevent them from widespread applications. Future attention may be paid in developing the fluorescent aptasensors as market products, and we expect that challenge will be eventually overcome. Finally, we appreciate the work reported on the fluorescent aptasensors for antibiotic detection and look forward to more mature aptasensors to be explored for market in the future. We hope this review gives readers an insight of the recent progress of this field, and inspires to develop new approaches for future studies.

Table 1. Summary of recent reported fluorescent aptasensors for the detection of antibiotics.

Sensing Strategy	Analyte	Fluorescent reporter	Linear range	LOD	Real sample	Ref.
G-quadruplex-based	Tetracycline	ThT	0.2-20.0 nM	0.97 nM	Serum (100-fold-diluted)	[24]
	Tetracycline	TO	0.05-100 μg mL^{-1}	0.029 μg mL^{-1}	Milk	[20]
	Kanamycin	TO	0.1-20 mM	0.059 mM	Milk	[23]
	Tetracycline	SYBR Green I	-	0.2 μg mL^{-1}	Milk	[13]
	Four tetracycline veterinary drugs : tetracycline,	NMM	0-1000 μg L^{-1} for all the four tetracycline	4.6 μg L^{-1} for all the four tetracycline	Milk	[25]

	oxytetracycline, chlortetracycline, doxycycline		drugs	drugs		
	Kanamycin	ThT	0.7-10 nM	0.37 nM	Milk	[26]
	Kanamycin	ThT	0.6-20 nM	0.33 nM	Milk	[27]
Liberation of cDNAs by analyte binding	Oxytetracycline, Kanamycin	FAM for oxytetracycline，ROX for Kanamycin NaYF4: Yb, Tm	1-50 ng mL^{-1} for oxytetracycline, 1-50 ng mL^{-1} for kanamycin	0.85 ng mL^{-1} for oxytetracycline, 0.92 ng mL^{-1} for kanamycin	Pork, milk, and honey	[30]
	Sulfadimethoxine	UCNPs	1-9 ng mL^{-1}	0.11 ng mL^{-1}	Fish	[28]
	Ampicillin	Taqman probe	0.1-100 ng mL^{-1}	0.07 ng mL^{-1}	Water	[29]
	Streptomycin	SYBR Gold	-	54.5 nM	Milk and blood serum	[12]
	Chloromycetin	CdSe QDs	0.05-100 ng mL^{-1}	0.002 ng mL^{-1}	Milk	[31]
	Chloromycetin	NaYF4: Yb, Er UCNPs	0.01-1 ng mL^{-1}	0.01 ng mL^{-1}	Milk	[18]
	Kanamycin	SYBR Gold	1-10 pg mL^{-1}	0.29 pg mL^{-1}	Milk and fish	[32]
Energy transfer	Kanamycin	Cy3/Cy5	1.0-80.0 nM	0.29 nM	Milk	[52]
	Enrofloxacin	Enrofloxacin	5-250 nM	3.7 nM	Milk	[53]
	Kanamycin	FAM	100-600 nM	13.52 nM	Milk	[21]
	Ampicillin	FAM	-	-	HEPES buffer	[54]
	Florfenicol	ATTO647N	5-1200 nM	5.75 nM	Milk	[55]
	Oxytetracycline	FAM	25-1000 μg L^{-1}	25 μg L^{-1}	Tap water and River water	[56]
	Kanamycin	CDs	4-25 μM	1.1 μM	Milk	[43]
	Oxytetracycline	FAM	0.01-0.2 μM	0.01 μM	Tap water and milk	[42]
	Chloramphenicol	$DSAC_2N$	0-100 ng mL^{-1}	0.00126 ng mL^{-1}	Milk	[41]
	Kanamycin	FAM	1-20 pM	1 pM	Serum and milk	[57]
	Sulfadimethoxine, Kanamycin, and Ampicillin	Cy3, FAM, and Cy5	-	1.997 ng mL^{-1} for sulfadimethoxine, 2.664 ng mL^{-1} for kanamycin, and 2.337 ng mL^{-1} for ampicillin	Milk	[22]
Others	Kanamycin	SYBR Green	0.5-10000 pg mL^{-1}	0.15 pg mL^{-1}	Milk and fish	[44]
	Kanamycin	RhB	24.75-137.15 nM	7.5 nM	Serum	[19]
	Chloramphenicol	SYBR Green I	0.1-20 ng mL^{-1}	0.1 ng mL^{-1}	Milk	[49]
	Kanamycin	CdSe QDs	0.05-10.0 μg mL^{-1}	0.013 μg mL^{-1}	Water, milk and urine	[45]
	oxytetracycline	Fluorescein	10-2000 nM	4.2 nM	Serum, plasma	[46]

Chloramphenicol	ThT	0.05-10 nM	0.016 nM	Milk, fish	[48]
Streptomycin	QDs	0.1-100 ng mL^{-1}	0.03 ng mL^{-1}	Milk	[47]
Tetracycline	EvaGreen	-	10 pM	-	[50]
Kanamycin	SYBR Green I	0.001-100 ng mL^{-1}	0.45 pg mL^{-1}	Milk, fish, pork	[51]

-: Data not available

Abbreviations: ThT: thioflavin T; TO: thiazole orange; NMM: Nmethylmesoporphyrin IX; FAM: carboxyfluorescein; ROX: carboxy-X-rhodamine; UCNPs: upconversion nanoparticles; QDs: quantum dots; Cy3: cyanine 3; Cy5: cyanine 5; CDs: carbon dots; RhB: Rhodamine B; $DSAC_2N$: 9,10-distyrylanthracene (DSA) derivative with short alkyl chains (9,10-bis{4-[2-(*N*,*N*,*N*-trimethylammonium)-ethoxy]styrene}anthracene dibromide)

Conflicts of interest

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

* * of outstanding interest

*Zhang, K., et al., Microfluidic electrophoretic non-enzymatic kanamycin assay making use of a stirring bar functionalized with gold-labeled aptamer, of a fluorescent DNA probe, and of signal amplification via hybridization chain reaction. Microchimica Acta, 2018. 185(3): p. 181.

The importance of this work lies in the involvement of signal amplification via hybridization chain reaction for enzyme-free aptamer-based assay.

**Sun, C.Y., et al., Label-free fluorescent sensor based on aptamer and thiazole orange for the detection of tetracycline. Dyes and Pigments, 2018. 149: p. 867-875.

The authors reported a typical G-quadruplex-based label-free fluorescent aptasensor for the detection of tetracycline, where the aptamer binding disrupted the G-quadruplex structure and released the fluorescent reporter thiazole orange. A significant point of the study is the use of CD spectra to confirm the binding-induced release of signaling dye.

* Zhou, C., et al., Fluorescent aptasensor for detection of four tetracycline veterinary drugs in milk based on catalytic hairpin assembly reaction and displacement of G-quadruplex. Analytical and Bioanalytical Chemistry, 2018. 410(12): p. 2981-2989.

Based on the nucleic acid nature of aptamer, the authors elaborated a catalytic hairpin assembly reaction for repeated use of analyte to amplify the reaction. Furthermore, this study simultaneously detected four tetracycline drugs by utilizing the multi-binding capacity of the selected aptamer.

*Ma, X.Y., et al., Development of Structure-Switching Aptamers for Kanamycin Detection Based on Fluorescence Resonance Energy Transfer. Frontiers in Chemistry, 2019. 7.

The article from Ma et al. is interesting, which reported the development of a typical structure-switching sensitive FRET mechanism between FAM fluorophore-labeled kanamycin-binding aptamer (FDNA) and a short oligonucleotide dabcyl quencher DNA (QDNA).

**Youn, H., et al., Aptasensor for multiplex detection of antibiotics based on FRET strategy combined with aptamer/graphene oxide complex. Scientific Reports, 2019. 9(1): p. 7659.

Article reported by Youn et al. is of outstanding interest due to the unique use of aptamer/graphene oxide complex that develops FRET through enzyme assisted cyclic enzymatic signal amplification. Most importantly, the system could rapidly detect three antibiotics simultaneously, giving it a significant edge in biosensing.

*Dehghani, S., et al., A label-free fluorescent aptasensor for detection of kanamycin based on dsDNA-capped mesoporous silica nanoparticles and Rhodamine B. Analytica Chimica Acta, 2018. 1030: p. 142-147.

The article reported an interesting label-free aptasensor based on mesoporous silica nanoparticles. The porous nanoparticles were capped with dsDNA and filled with Rhodamine B. Aptamer DNA would be separate from complementary strand in the presence of analyte. As a consequence, the Rhodamine B released from uncovered pores.

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