Current trends in nanomaterials-mediated biosensing platforms and signal amplification strategies for antibiotics detection in dairy products

Cui-Yun Zhou, Feng Jiang, Chen-Xi Huang

1 Hubei Provincial Institute for Food Supervision and Test, Wuhan 430070, China. 2 College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China. 3 Key Laboratory of Detection Technology of Focus Chemical Hazards in Animal-derived Food for State Market Regulation Wuhan 430070, China. 4 Hubei Provincial Engineering and Technology Research Center for Food Quality and Safety Test, Wuhan 430070, China. 5 State Key Laboratory of Biocatalysis and Enzyme Engineering, Hubei Key Laboratory of Industrial Biotechnology, School of Life Sciences, Hubei University, Wuhan 430062, China.

*Corresponding to: Chen-Xi Huang, College of Food Science and Technology, Huazhong Agricultural University, No.1 Shizishan Street, Hongshan District, Wuhan 430070, China. E-mail: huangchenxi@mail.hzau.edu.cn

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Cuyun Zhou: Investigation, Methodology, Formal Analysis, Writing - Original Draft; Feng Jiang: Formal analysis, Supervision, Funding Acquisition.; Cheni Huang: Conceptualization, Writing - Review & Editing, Supervision, Funding Acquisition.

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Abbreviations
ALP: Alkaline phosphatase; CBPV: Community board for plant varieties; DLISA: Decoyrylzyme-based enzyme-linked immunosorbent assay; ECL: Electrochemical luminescence EFSA: European food safety authority; ELISA: Enzyme-linked immunosorbent assay; FRET: Fluorescence resonance energy transfer; FVO: Food and veterinary office; HPLC: High-performance liquid chromatography; HRP: Horseradish peroxidase; IN: Invertase; LOD: Limit of detection; MOF: Metal-organic framework; MRLs: Maximum residue limits; RSD: Relative standard deviation.

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Abstract
Dairy products have become one of the most prevalent daily foods worldwide, but safety concerns are rising. In dairy farming, unscrupulous traders misuse antibiotics to treat some diseases such as mastitis in cows, leading to antibiotic residues in dairy products. Rapid, sensitive, and simple detection methods for antibiotic residues are particularly important for food safety in dairy products. Traditional detection technology can effectively detect antibiotics, but there are defects such as complicated pre-treatment and high cost. Biosensors are widely used in food safety due to fast detection speed, low detection cost, strong anti-interference ability, and suitability for the field application. Nevertheless, these sensors often fail to trigger the signal conversion output due to low target concentration. To cope with this issue, some high-efficiency signal amplification systems can be introduced to improve the detection sensitivity and linear range of biosensors. In this review, we focused on: (i) Sources and toxicity of major antibiotics in animal-derived foods. (ii) Nanomaterial-mediated biosensors for real-time detection of target antibiotics in animal-derived foods. (iii) Signal amplification techniques to increase the sensitivity of biosensors. Finally, future prospects and challenges in this research field are discussed.

Keywords: Nanosensors; Signal amplification; Antibiotics detection; Animal-derived foods.
Introduction

In recent years, there has been an increase in the demand and consumption of dairy products worldwide. The production volume and total output value of dairy products have experienced significant growth over the recent decades [1]. Dairy products are the most important foodstuffs in the daily consumption of animal-source foods. However, safety incidents caused by antibiotic residues in dairy products frequently occur, and the safety of dairy products has become a widely discussed topic of public concern [2–4]. Pollutants in dairy products mainly include biological pollutants like harmful microorganisms and biological hormones [5, 6], and chemical pollutants such as heavy metals, pesticide and veterinary drug residues, fungal toxins and illegal chemical additives [7, 8]. Other illegal additives found in dairy products are protein essence, melamine, sodium thiocyanate, leather hydrolysate, β-lactam, leather hydrolysat, and β-lactamase [9, 10]. Among the pollutants, antibiotics are one of the most harmful pollutants [11, 12].

Antibiotic residues may not only pose risks to the health of consumers, but also inhibit the normal microflora in milk. Thus there are potential adverse effects on the manufacturing processes of some dairy products [13]. On the one hand, antibiotic-containing dairy products cause allergic reactions from mild to severe, which can be life-threatening in some severe cases [14–16]. Moreover, foods with low residual levels of antibiotics can cause antibiotic resistance in humans, leading to microenvironment imbalances and the occurrence of drug-resistant bacteria. Therefore, it poses challenges to the treatment of human diseases [17]. Under this condition, a disruption of normal intestinal microflora can lead to severe disease by causing secondary infections in the human body. On the other hand, antibiotic in dairy products could interfere in the fermentation process through the inhibition of starter cultures used in the production of cheese, yogurt, and butter, therefore resulting in economic losses [18, 19]. Additionally, the existence of some antibiotics is stable in the natural environment and can cause environmental pollution and destroy the ecological balance [18, 20, 21].

Antibiotics contaminate milk directly or indirectly during the processing and preservation of milk and related dairy products [22–24]. The main sources are: (i) Feed contamination: Dairy farmers add antibiotics to feed to treat diseases such as mastitis and gastrointestinal problems in dairy cows. Unsuspicious dairy farmers use large amounts of antibiotics to achieve immediate results, resulting in the accumulation of antibiotic residues in milk products. Therefore feed contamination will undermine human health [25]; (ii) Intramammary injections: Mastitis is one of the most common diseases in dairy cows. In dairy cows, dairy farmers inject a wide range of antibiotics to treat and prevent mastitis. This method is fast and effective, but it can easily lead to antibiotic residues in the milk [26]; (iii) Antibiotics as an additive in milk: Milk is sometimes not delivered to the dairy in time and is not stored properly. Antibiotics were directly added to milk by some unsuspicious people to prevent contamination [27]; (iv) Contamination of tools and storage equipment during the milk processing: Milking equipment and storage tools used on sick cows contaminate milk with antibiotics due to inadequate cleaning and sterilization before using on healthy cows. Dairy cows inevitably get diseases during their feeding, therefore antibiotics are required and treated [28, 29]. Antibiotic residues in dairy products can be greatly reduced, if dairy farmers strictly observe the withdrawal period and other relevant regulations when using antibiotics. Based on this, the supervision of the overseer of antibiotics needs to be strengthened.

Antibiotics have significant effects on the treatment of bacterial infectious diseases in humans and animals. However, the overseer of antibiotics has led to increasing concerns about their residues in food (Figure 1). Some antibiotics can be used as veterinary medicines for the treatment of mastitis in dairy cows which will result in antibiotic residues in the milk when improperly used. Residues of antibiotics in milk can seriously affect consumption, and human health, causing allergic reactions and the production of drug-resistant strains after long-term use [30]. The alarming misuse of antibiotics can cause increasing environmental pollution and antibiotic resistance [31, 32]. The World Health Organization warns that by 2050, the issue of antibiotic contamination in dairy products will become increasingly severe [33]. Based on this, it is necessary to propose and implement control measures and testing methods as early as possible.

Structural characteristics and hazards of major antibiotics in dairy products

Antibiotics commonly used in dairy cows include injectable drugs (such as penicillin G, erythromycin, gentamicin, ampicillin, streptomycin sulfate) and internal drugs (such as tylosin, hygromycin hydrochloride, tetracycline hydrochloride) [34]. Considering the relevant standards at home and abroad as well as practical applications, chloramphenicol, kanamycin, neomycin, streptomycin, gentamicin, and tetracycline were selected as the representative targets in this study [35]. The maximum residue limits (MRLs) of these antibiotics in milk are shown in Table 1. The detection methods used in these standards are mainly enzyme-linked immunosorbent assay (ELISA), color-assay spectrometry (including gas chromatography, liquid chromatography, and mass spectrometry), and microbiological methods.

Chloramphenicol

Chloramphenicol is a broad-spectrum amphenicol known for its strong antibacterial properties, with a chemical formula of C18H19Cl2N2O. It is highly stable and resistant to high temperatures but loses its effectiveness under extremely alkaline conditions. Chloramphenicol interferes with the function of transpeptidases and inhibits the formation of bacterial peptide chains and protein synthesis, so it is commonly used in animal husbandry to treat bacterial infections in animals [36]. However, it is important to be aware of the toxic and side effects associated with chloramphenicol due to its para-nitro structure. Toxicological data in the literature using mice indicate that the nitrobenzene ring in the structure of chloramphenicol is potentially toxic and can cause irreversible myelosuppression in organisms [37]. Moreover, chloramphenicol is highly stable and its toxicity persists in the environment for long periods. Chloramphenicol residues can be metabolized and lead to allergies, neurotoxicity, reproductive system diseases, and even leukemia [38]. Hence, it is necessary to monitor chloramphenicol residues in milk to ensure the safety of consumers.

Kanamycin

Kanamycin is a protein synthesis inhibitor that effectively treats various bacterial infections. It is an important subclass of aminoglycoside antibiotics and could effectively prevent infections from bacteria such as Bacillus, Staphylococcus, and Escherichia coli. Therefore, it is widely used in the treatment of severe infections in dairy cows with remarkable efficacy. However, excessive use of kanamycin as an antibiotic can result in its residues in dairy products. Consuming dairy products containing kanamycin can lead to adverse effects on the human body, including ototoxicity, nephrotoxicity, blockade of neuromuscular junctions, anaphylactic reactions, and even death, thus posing a serious threat to human health [39]. As a result, kanamycin contamination has become a significant global food safety issue. It is crucial to take effective measures to reduce the use of kanamycin, strengthen food testing and regulation, and ensure the safety and health of the food consumed by people.

Neomycin

Neomycin is isolated by fermentation and extraction from the Streptomyces genus Streptomyces fusiformis, one of the most toxic aminoglycoside antibiotics. It is alkaline and extremely soluble in water. Its sulfate salt, also known as neomycin sulfate, is commonly used in the market. It exerts its bacteriostatic effect mainly by binding to the 30S ribosomal subunit, leading to RNA misinterpretation and thus impeding bacterial protein synthesis. It has good inhibitory effect
on most gram-negative bacteria, some gram-positive bacilli and leptospirosis. Neomycin sulfate is chemically stable, inexpensive, and has obvious bacteriostatic effect, and is one of the first choice drugs for the treatment of intestinal bacterial infections in livestock and poultry. It can be used to treat diseases such as mastitis in dairy cows. However, overuse of neomycin by unscrupulous farmers can lead to residual neomycin in dairy products. Neomycin has been proven to cause toxicity in humans such as neurotoxicity, brain nerve damage, irreversible hearing loss, kidney damage, loss of appetite, nausea and vomiting [40, 41].

Streptomycin
Streptomycin sulfate is an aminoglycoside antibiotic, an antibiotic extracted from the culture of Streptomyces griseus. Streptomycin has a strong antibacterial effect on Mycobacterium tuberculosis, mainly binds to the 30S subunit of bacterial ribosome and interferes with bacterial protein synthesis to achieve the effect of bacterial inhibition. Toxic reactions including numbness, dizziness, deafness is common, but also occur in perioral numbness, dizziness, dyskinesia, headache, fatigue, vomiting, and facial flushing, ototoxicity is similar to gentamicin [42]. In addition to local irritation, other symptoms mainly include intramuscular injection of local pain, swelling, aseptic abscess. The damage to the kidneys damage is less severe, manifested as proteinuria and tubularia, and some of them show temporary renal decompensation [43].

Gentamicin
Gentamicin is an aminoglycoside antibiotic produced by the fermentation of Micromonospora purpurea, a multi-component antibiotic primarily used to treat infections caused by bacterial Gram-negative bacteria. The inhibitory substrate of gentamicin is that it can bind to the 30S subunit of the bacterial ribosome, interrupting the replication of RNA, interfering with the central law, and denying the bacterium proteins leading to its death. Adverse reactions (including systemic application combined with intrathecal injection) can cause leg cramps, rash, fever and generalized spasm, a higher incidence of hearing loss, ototoxicity, hematuria, significant reduction in the frequency or volume of urination, loss of appetite, and nephrotoxicity, especially in neonates, the elderly, and patients with renal insufficiency [44–46].

Tetracycline
Tetracycline is a kind of antibiotic extracted from the culture fluid of actinomycetes, which has a good inhibitory effect on bacteria, rickettsiae, filtration viruses, spirochete genera, and even protozoa. It is a kind of broad-spectrum antimicrobial agent, but not effective against tuberculosis bacteria and aspergillus. The mechanism of bacteriostatic action of tetracycline is that the drug prevents the chaining of aminocyl-tRNA in the A position of the ribosomal subunit, thus inhibiting the extension of the peptide chain and affecting the central law, breaking the bacterial proteins and leading to the death of the bacteria without nutrients. It can also cause side effects including liver toxicity, usually fatty liver degeneration, purpura, anaphylaxis, and asthma [47, 48]. After long-term exposure, it could also cause hemolytic anemia, thrombocytopenia, neutropenia, eosinophilia, and nephrotoxicity. The occurrence of drug-resistant Staphylococcus aureus, gram-negative Bacilli, and fungi can then cause gastrointestinal, respiratory, and urinary tract infections [49].

Table 1 The MRLs of five antibiotics in milk stipulated by relevant standards at home and abroad (μg/kg)

<table>
<thead>
<tr>
<th>Target</th>
<th>Structure</th>
<th>China</th>
<th>EU</th>
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<tr>
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<td>![Structure](Image 216x187 to 274x241)</td>
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<td>150</td>
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<tr>
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<td>200</td>
<td>150</td>
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<tr>
<td>Gentamicin</td>
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<td>30</td>
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<tr>
<td>Tetracycline</td>
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Advances in the detection of antibiotics in dairy products

Classical antibiotic detection
To detect antibiotic residues in dairy products, various methods have been developed to detect antibiotics. The classical detection methods have mainly relied on high-performance liquid chromatography (HPLC) [53, 54], ELISA [55, 56], and capillary electrophoresis [57, 58].

HPLC is the most widely used analytical technique for antibiotic assays in different samples. For instance, a sensitive and specific HPLC method has been developed for the first time to simultaneously quantify the six tetracyclines in dairy products [59]. The acetonitrile-ultrafast microextraction method was used for sample pretreatment, and then a gradient reversed-phase HPLC method was used for the simultaneous determination of six tetracyclines in a variety of dairy products. The analytical time of the method was 9 min, with good peak resolution and peak symmetry within the elution time. It has the advantages of short analysis time and high analytical efficiency. The recovery rate of dairy products is above 80%, indicating that the optimized method has good stability and repeatability. However, HPLC greatly relies on large-scale precision instruments and professional operation technical personnel, thus greatly restricting their practicability.

ELISA is a widely used analytical method with the advantages of simplicity, low analytical cost, sensitivity, specificity, and rapidity of detection, as well as the ability to measure multiple samples simultaneously. Horseradish peroxidase (HRP), a key enzymatic component of ELISA, amplifies the detection signal [60]. In some studies, artificial deoxyribonuclease was used to replace the traditional horseradish peroxidase, and the proposed immunoassay was optimized to detect chloramphenicol with high sensitivity and specificity under optimal conditions, with a linear range of 1.0 pg/mL-1.0 µg/mL, and a limit of detection (LOD) of 0.1 pg/mL, which is much lower than that of the traditional ELISA of 40 pg/mL-100 pg/mL [61-63]. The recoveries in milk were 86% - 104%. It showed that the ELISA method has a wide linear range and good practical performance. However, the detection process includes multiple spiking, incubation and repeated washing procedures, which is cumbersome but not as reproducible as precision instruments such as HPLC. Therefore, there is an urgent need for more advantageous assays in the market.

Capillary electrophoresis uses a high-voltage direct current electric field as a driving force for analyte separation performed in capillaries. Despite its inherent advantages, the sensitivity of capillary electrophoresis is not high enough and restricts its application in monitoring pesticides, veterinary drugs, and toxins, developed microfluidic chip-based capillary electrophoresis system via enzyme-free dynamic DNA amplification for sensitive detection of chloramphenicol and kanamycin, with LODs of 1.61 amol/L and 0.7 amol/L, respectively [64]. The microfluidic electrophoresis analysis system can greatly improve detection speed and sensitivity, but it still suffers from expensive equipment and high cost in capillary electrophoresis.

Among detection approaches, these detection methods rely on cumbersome operation processes, long detection times, high equipment cost, and professional personnel to operate, strictly limiting its large-scale practical application. With the development of the combination of biotechnology and detection technology, biosensors have been widely used in the detection of antibiotic residues based on the conversion of signals from specific bio-recognition elements [65]. Biosensors are analytical tools that can provide sensitive, reliable, rapid, and portable tools for monitoring and point-of-care testing of antibiotics. Biosensors-mediated methods for antibiotic detection have been approved by the Food and Veterinary Office (FVO), the European Food Safety Authority (EFSA), and the Community Board for Plant Varieties (CBPV) [66]. A biosensor combines a signaling probe (typically biocompatible) with a specific analyte of interest to produce an intuitive, measurable signal, the intensity of which is linearly related to the analyte concentration. The function of the transducer in the process is to convert the primary signal generated by the recognition element into a measurable form that can be used to represent, store, analyze, and even amplify the signal. With the transducer, signals can be converted to include electrochemical, fluorescent, and optical signals, and the concentration of the analyte can be deduced from the captured signal. Due to the advantage of strong specificity, high sensitivity, simplicity, and portability, biosensors were used for antibiotic detection [67]. According to the detection principle, biosensors can be divided into electrochemical biosensors, optical biosensors, and other types.

Nanomaterial-mediated biosensors detection
Biosensors work on a sensing mechanism that converts the interaction response of a biological analyte into a recognizable signal form. Nanomaterials were signal probes with excellent performance and have enabled biosensors to detect target analytes more efficiently because of their advantages such as high surface-to-volume ratios, good dispersion and biocompatibility properties [68]. Therefore, nanomaterial-mediated biosensors provide a new and advanced...
platform in the field of rapid detection. The intervention of nano-engineered materials changes the performance of the sensors such as sensitivity, specificity, response time, reagent volume and device cost [69]. Furthermore, nanomaterials were used to develop a range of no-wash electrochemical and optical sensors [70]. For example, wash-free electrochemical biosensors can be constructed based on covalent interactions between nanomaterial coatings and labeled probe electrodes, while wash-free and separation-free optical sensors can be constructed based on the unique optical properties or homogeneous reaction characteristics of labeled probes. These biosensors do not require repetitive and laborious washing or purification steps to remove matrix interferences, background and unbound labeled probes during the detection process [71].

With the emergence of various nanomaterials and the development of different biosensing strategies, it has been found that the crux of the problem lies in the selection of stable nanomaterials in order to construct a fast, stable platform for antibiotic detection. This paper reviews the research progress of nanomaterial-based biosensors for the detection of antibiotics in dairy products. And also summarizes the common signal amplification mechanisms applied in biosensors for rapid, sensitive and reliable determination of antibiotics.

**Nanomaterial-mediated voltammetric electrochemical sensors.**

Electrochemical biosensors have especially great potential for widespread use in the shortest time and the least cost of instruments can be reduced. For example, Zhang et al. proposed a simple, highly sensitive, and low-cost magnetic molecularly imprinted particle electrochemical biosensor for the simultaneous detection of multiple antibiotics. They used metal ions as signal tracers and amplifiers, and magnetic molecularly imprinted polymers modified glassy carbon electrodes. The peak current in response to metal oxidation was then recorded using differential pulse voltammetry and used to monitor the levels of aminoglycoside antibiotics. Under optimal conditions, the electrochemical sensor based on magnetic molecularly imprinted particles showed high sensitivity for kanamycin, tobramycin and gentamicin with LODs of 4.88, 1.28 and 1.07 nmol/L, respectively [83]. Li et al. developed a molecularly imprinted electrochemical sensor for the detection of streptomycin. A molecularly imprinted polymer was prepared by electropolymerization using graphene oxide and chitosan composites to modify the electrodes, with streptomycin as the template and 3-aminophenylboronic acid and aniline as the monomers. The sensor showed a high binding affinity and selectivity towards streptomycin at the LOD of 2.54 × 10^{-11} mol/L. The molecularly imprinted sensing interface showed a high binding affinity and selectivity towards streptomycin. The sensor could rapidly and accurately detect streptomycin in milk samples with recoveries of 89.46% - 101.2%. This method has shown good reproducibility, stability, and regeneration ability [84]. However, electrochemical biosensors suffer from poor sensitivity and stability, and low reproducibility. Therefore, it is of great significance to further explore new composite materials such as nanomaterials or carbon-based materials with good biocompatibility and conductivity to modify electrodes.

**Nanomaterial-mediated electrochemical luminescence.**

Electrochemical luminescence (ECL) combines electrochemical and luminescence techniques, which provide higher selectivity and sensitivity [85, 86]. Compared with voltammetric electrochemical sensors, electrochemical luminescence was developed based on chemiluminescent agents and electron donors, which simultaneously lose their electron oxidation reaction on the cathode surface to form strong oxidants and cationic radicals. The two highly active groups quickly reacted on the electrode surface and emitted photons to generate signals [87-89].

Due to the limited electron transfer in ECL, the sensing efficiency is low, and methods such as nanomaterials can be used to amplify luminescent signals. Zhao et al. developed a novel “on-off-on” system for the highly sensitive determination of kanamycin on the ECL platform (Figure 4). Furthermore, a three-layer composite film is used

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to modify the glassy carbon electrode, with the first layer wrapped with C60 nanoparticles modified with gold nanoparticles. The middle layer is poly L. histidine hydrochloride, and the outermost layer is self-assembled colloidal gold nanoparticles. This three-layer structure can significantly amplify ECL signals through covalent bond interactions. As for detecting kanamycin, the capture probe is fixed on a three-layer composite film-modified electrode. The ECL signal can be quenched and detected in an "off" state, after DNA hybridization. The developed "on off on" system detects kanamycin with a linear range of 0.15 nmol/L-170 nmol/L, reaching 9 orders of magnitude and the LOD of 45 pmol/L. [90]. An ultrasensitive detection electrochemiluminescence immunoassay for chloramphenicol was developed using modified hollow titanium dioxide and SnS2 quantum dots. Hollow spheres of titanium dioxide modified with polyethyleneimine and gold nanoparticles were synthesized to serve as signal probes and also as promoters of synergistic reactions, which greatly improved the interaction efficiency of the quantum dots. It was used for streptomycin detection with high sensitivity, the linear range is 0.01 ng/mL - 100 ng/mL, and the LOD is as low as 3.1 pg/mL [91]. Functionalized MOF is uniquely advantageous in the field of sensing because of its wide range of functionalities. It is possible to design biotarget electrochemiluminescent molecularly imprinted sensors that employ Zn-MOF signaling probes with biluminescent properties. The dual luminescence signal is more sensitive and stable than the single signaling luminescent moiety usually used, providing reliability and accuracy for the detection of trace levels of chloramphenicol. Under the optimal conditions, the LODs were 2.1 fmol/L and 2.5 fmol/L, respectively. The proposed biluminescent sensing system is suitable for the sensitive detection of trace antibiotics, and has been widely used in food safety field [92].

**Nanomaterial-mediated optical biosensor.** Optical biosensors are a class of sensors that utilize light absorption, reflection, refraction, fluorescence and luminescence to analyze and detect targets. They have the advantages of high detection sensitivity, low cost, fast speed and field application, and are widely used in the fields of food safety, biomedicine, healthcare, environmental monitoring, etc [93]. Among them, fluorescent biosensors and colorimetric biosensors are the most commonly used biosensors.

Fluorescent biosensors can be divided into fluorescence spectroscopy and fluorescence resonance energy transfer (FRET), which have unique advantages of high sensitivity and efficiency [94]. The principle of the former is that different substances absorb the energy of light under ultraviolet light and emit fluorescence spectra of different wavelengths. The concentration of a specific target substance is determined by determining the relationship between fluorescence intensity and target substance concentration. FRET is based on the overlap of the emission spectrum of one fluorescent substance with the absorption spectrum of another substance. The latter absorbs the energy of the former and emits light, and monitors the concentration of the former by measuring the emission spectrum of the latter. Currently, quantum dots, fluorescent dyes, and nanomaterials with fluorescence characteristics are commonly used to establish fluorescence spectroscopy detection methods. Fan et al. designed a novel fluorescent sensor based on thiosuccinic acid-modified quantum dots to achieve visual and rapid detection of aminoglycoside antibiotic neomycin (Figure 5). With the increase in neomycin concentration, the fluorescence changes from bright yellow to dark red. The sensor exhibits excellent neomycin detection performance with the LOD of 16 nmol/L, a recovery rate of 95.66% - 100.77% in actual samples, and an RSD of less than 3.15%, with high accuracy and repeatability [95]. A simple, rapid and efficient fluorescence detection method for chloramphenicol has been established by utilizing the fluorescence property of silicon quantum dots and the excellent rupture ability of oxidized single-walled carbon nanotubes. The single-walled carbon nanotubes were coupled with carboxyl groups to form a complex with good dispersibility, and the detection of chloramphenicol was achieved in different real samples with the LOD of 0.6 mg/mL and a recovery of 94.1%. This method can accurately determine the content of chloramphenicol in milk samples with RSD 6.1% [96]. Wu et al designed a novel fluorescent switch sensor to detect streptomycin using single-stranded DNA-binding protein and exonuclease I-labeled quantum dots. A fluorescent probe was synthesized by labeling the quantum dots with single-stranded DNA-binding proteins, which can capture the aptamer. When hybridized with the aptamer, the quantum dots can be aggregated in solution and burst the quantum dots, and the state of the sensor changes from "on" to "off". In the presence of streptavidin and exonuclease I, the aptamer can bind to the target. Nuclease I then digest the aptamer into individual nucleotides. The released target can participate in the reaction cycle, producing a strong fluorescent signal. At this point, the distance between the quantum dots increases, thus restoring the fluorescence intensity. Therefore, the switch changes from the "off" state to the "on" state, and the linear range of streptomycin detection is 0.1 ng/mL - 100 ng/mL, with a LOD of 0.03 mg/mL [97].

The combination of nanomaterials that can generate energy transfer is commonly used in FRET. Wang et al. constructed a novel fluorescence sensor based on the principle of FRET, using hairpin-shaped DNA silver nanoclusters and core-shell gold-palladium nanoparticles regulated by guanine bases. Illuminating DNA silver nanoclusters with weak fluorescence emission and adding different amounts of guanine bases to the hairpin ring can cause the emission spectrum to shift red and increase the fluorescence intensity of DNA silver. Gold-palladium nanoparticles and core-shell structure were prepared by seed growth and deposition, which improved the bursting ability of palladium nanoparticles in the long-wave range and significantly expanded the UV absorption range of gold nanoparticles. The linear range of the sensor for the detection of streptomycin was 50 nmol/L - 1250 nmol/L, and the LOD was 18.7 nmol/L. In addition, this strategy has been successfully applied to the detection of streptomycin in milk [98]. An ultrasensitive FRET sensor was developed using upconversion nanoparticles and graphene as energy donors and acceptors for kanamycin detection, respectively. Oleic acid-modified upconversion nanoparticles were synthesized by exchanging ligands with adipic acid using a hydrothermal method. The ultra-high sensitivity and remarkable robustness of this sensor in complex sample matrices offer good prospects for practical applications, with LOD of 9 pmol/L for kanamycin [99]. By using this FRET pair composed of fluorescent carbon dots and layered molybdenum disulfide, kanamycin can be quantified in the concentration range of 4.25 µmol/L, with a LOD of 1.1 µmol/L, providing a promising tool for the rapid detection of kanamycin in milk and other animal-derived foods [100]. Due to the advantages of high sensitivity and wide linear range, fluorescent sensors have good application prospects in the field of food safety for detecting antibiotics. However, fluorescent reagents are usually expensive and have high storage conditions. Therefore, exploring low-cost optical reagents combined with simple optical detection methods is of great significance.

Colorimetric sensors allow quantitative detection of analytes based on the different color changes that result from the reaction of different concentrations of analytes with color-developing reagents [101]. The commonly used colorimetric sensors first establish a standard curve based on the absorbance of gradient concentration solutions and then substitute the absorbance of the substance to be tested into the standard curve to achieve quantitative analysis. At present, the commonly used fluorescence detection instruments for antibiotic detection mainly include ultraviolet visible spectrophotometer and enzyme-linked immunosorbent assay.

The interaction between biosynthesized silver nanoparticles and kanamycin led to the development of a colorimetric sensor. silver nanoparticles solution changed from yellow to reddish-pink in the presence of kanamycin with a concomitant decrease in the intensity of the yellow band. The results showed that the method was sensitive with the LOD of 0.65 µmol/L for colorimetric detection of kanamycin [102]. In addition, combining nanomaterials can further improve the sensitivity of colorimetric sensors. Zhao et al introduced MOF as a carrier for high-capacity loaded HRP-labeled immunoglobulins and
gold nanoparticles to prepare MOF hybrids with enhanced peroxidase activity (Figure 6). The prepared MOF hybrid was used for colorimetric detection of chloramphenicol by establishing an indirect competitive immunooassay, with a linear range of 8 ng/mL to 108 ng/mL and a LOD of 6 pg/mL. The recovery rate of the spiked milk sample was 76.0% -106.0%, but the usage of antibodies and antigens was only 1/5 of that of conventional enzyme-linked immunosorbent assay, which to some extent saved detection costs. The proposed colorimetric sensor using MOF hybridized immune probes provides a new platform for the ultra-sensitive determination of chloramphenicol residues [103].

In summary, biosensors have the advantages of high sensitivity, fast detection speed, and short response time compared to traditional methods, and are widely used in the detection of antibiotic residues. However, there are still many shortcomings in practical applications. For example, in electrochemical biosensors, a small number of targets can cause changes in the signal, resulting in high sensitivity. However, it is precise because electrochemical biosensors are also susceptible to external environmental factors [104]. Fluorescent biosensors derive their signals from the fluorescent properties of substances. However, the disadvantage that fluorescence is easily burstable poses a challenge to fluorescent biosensors [105]. The sensitivity of optical biosensors based on colorimetric analysis varies depending on the different extinction coefficients of different substances [106, 107]. Therefore, the development of new biosensors combining efficient signal amplification and signal readout platforms to improve their performance in practical detection is still an urgent problem to be solved.

**Signal amplification strategy in the detection of antibiotics in dairy products using biosensors**

**Concept and principle of signal amplification system**

The basic principle of biosensors is to use the linear relationship between the concentration of the target analyte and the intensity of the readout signal to quantitatively analyze the target. However, in practical applications, there are some issues that the target concentration is too low to trigger the signal conversion function of the transducer [108]. An efficient signal amplification system is utilized to solve this problem. Increasing the intensity of the corresponding output signal when the concentration of the target substance is constant can dramatically improve the sensitivity and linear range of the biosensor. Currently, commonly used signal amplification techniques mainly include enzyme-catalyzed, click chemistry, cascade reactions, and biotin chain affinity-mediated signal amplification strategies [109].

**Enzymatic-mediated signal amplification strategy**

Enzymes are made of a protein or RNA with high selectivity and catalytic properties for substrates. Utilizing this property to catalyze the corresponding substrate and convert undetectable signals into strong signals for readout is an effective way to improve sensitivity. Currently, the commonly used catalytic enzymes are HRP, alkaline phosphatase (ALP) and invertase (INT) [110].

The signal amplification technology based on enzyme catalysis has been widely applied in biosensors for detecting antibiotics in food. For example, an ALP-modulated fluorescent sensor was developed. The Fe₃O₄@COF probes synthesized by the hydrothermal method have the dual function of magnetic carrier and signaling probe for chloramphenicol detection. Bovine serum albumin coupled with chloramphenicol adsorbed on the surface of Fe₃O₄@COF binds to a chloramphenicol-competitive antibody. The antibody interacts with ALP through the biotin-affinity system. Meanwhile, the ascorbic acid produced by the enzyme-catalyzed reaction dominated by ALP could effectively restore the fluorescence of Fe₃O₄@COF burst by Fe₃O₄. The method has good stability (15 days) and reusability (8 cycles), providing a sensitive and reliable method for the accurate detection of chloramphenicol, with the LOD of 0.092 ng/mL [111]. Artificial deoxyribonucleases were used instead of HRP (or ALP) to generate amplification signals in enzyme-linked immunosorbent assays. The feasibility of the deoxyribozyme-based enzyme-linked immunosorbent assay (DLISA) was developed for the detection of chloramphenicol, which is a banned veterinary drug (Figure 7). The results showed that the DLISA was highly sensitive in the detection of chloramphenicol at the LOD of 0.1 ng/L. A wide linear range of 1.0 pg/mL-1000 ng/mL was achieved with the spiked recoveries of 86%-104%, which proved to be well-performed. In addition, DLISA antibody binds easily to DNAzyme through the simple functionalization process of gold nanoparticles [112].

**Click chemistry-mediated signal amplification strategy**

Click chemistry was first proposed by Kolby in 2001 [113], which is a chemical synthesis reaction with special advantages compared to traditional reactions. By splicing various small units, different types of molecules can be quickly and reliably formed. Click chemistry is one of the orthogonal reactions that has high specificity and can react quickly in aqueous solutions at room temperature with almost no by-products [114]. Therefore, compared to conventional reaction modes, the most prominent advantage of click chemistry is its high selectivity and efficiency, which is a fast, flexible, and highly selective biological reaction. In addition, ligands in click chemistry are small molecules that can be easily modified on biological macromolecules (e.g., proteins, antibodies, enzymes, and nanoparticles) and used in biosensors to achieve signal amplification.

There are currently four types of click chemistry: nucleophilic ring-opening reactions, cycloaddition reactions, non-aldehyde carbonyl reactions and carbon-carbon double bond addition reactions. Among them, the most widely used is the copper-catalyzed cycloaddition of alkenes and azides. Click chemistry in which the size of the ligand is very small and the modification process does not interfere with the biological activity of the labeled molecule. Based on these advantages, click chemistry is widely used in different functional biosensors, Xianyu et al. developed a novel magnetic relaxation switch sensing method, which uses tetrazine trans cyclooctene click chemical assembly of latex microspheres and magnetic nanoparticles of different sizes to prepare multifunctional magnetic probes with high sensitivity and wide linear range. By using click chemistry, small magnetic nanoparticles can be controllably assembled onto latex microspheres of different sizes, forming a core-satellite structure and constructing highly sensitive magnetic relaxation switch biosensors. In addition, latex microspheres of different sizes can combine with different numbers of small magnetic nanoparticles, making the magnetic relaxation switch sensor have an adjustable linear range, which can be analyzed from pg/mL to pg/mL of various antibiotics [115]. A molecularly imprinted membrane extraction method combined with UHPLC-MS/MS analysis was developed for the simultaneous selective identification and detection of chloramphenicol residues in complex matrices. The molecularly imprinted membranes can be prepared in a short time under mild reaction conditions by using bifunctional monomers, methacrylate, and acrylamide, with a “click chemistry” polymerization strategy. The molecularly imprinted membrane is characterized by good surface hydrophilicity, excellent selectivity, and the ability to exclude macromolecules. Finally, a rapid, accurate, and feasible analytical method was developed for the sensitive identification and quantification of trace amounts of chloramphenicol in milk (40 ng/kg-290 ng/kg) by UHPLC-MS/MS analysis [116].

**Cascade reactions mediated signal amplification strategy**

Cascade reaction refers to the triggering of multiple reactions in the same reaction system through a certain medium. The advantages are: (i) further enhancement and amplification of the signal based on the multiple steps of the cascade reaction; (ii) conversion of a single reaction that cannot trigger a signal conversion into a cascade reaction of detectable signals; and (iii) conversion of the signal generated by the original single reaction mode into another signal output with higher sensitivity after a multi-step reaction. Currently, cascade reactions are commonly used with multi-enzyme catalyzed reaction
systems and signal output conversion systems for signal amplification, and combining cascade reactions with biosensors for detection and analysis is an effective means of detection.

Ye et al. developed a ratio fluorescence platform, which provides a built-in self-calibration function for signal correction and can eliminate interference caused by target-independent factors. It is used for kanamycin measurement mediated by enzyme-free cascade signal amplification. Using catalytic hairpin components and chain displacement coupling driven by deoxyribonuclease to construct a cascade reaction. To build a detection platform, two fluorescent groups were inserted into the upstream and downstream reactions, respectively. Once the cascade reaction is activated, through DNA hybridization and deoxyribonuclease cleavage, the two fluorescent groups exhibit reversible signal changes that can be simultaneously recorded by synchronous spectroscopy. Under the optimal reaction conditions, this cascade signal amplification mediated sensor detected kanamycin with the LOD of 21.71 pmol/L, and achieved the determination of kanamycin in milk, indicating the potential application value of this enzymatic cascade signal amplification method with proportional signal output in antibiotic detection [117]. A fluorescent detection system for chloramphenicol based on dumbbell DNA-mediated signal amplification was developed (Figure 8). Two hairpin dimers were used as building blocks to construct the sensing scaffold. Highly fluorescent signals were generated in the products formed by the cascade DNA ladder for chloramphenicol monitoring. The dimeric hairpin assemblies showed improved signal amplification efficiency and shorter reaction time compared to the monomeric hairpin assemblies. The developed chloramphenicol sensor has a wide linear range from 10 fmol/L to 10 nmol/L with a LOD of 2 fmol/L. Importantly, the sensing platform has been successfully applied for the determination of milk chloramphenicol with satisfactory recovery and accuracy [118].

Figure 2 Schematic of chloramphenicol detection based on AgNP/[NH2-Si]-f-GO. Reprinted with permission from Ref. [74]

Figure 3 Principle of electrochemical sensor based on UiO-66-NH2. Reprinted with permission from Ref. [80]
Figure 4 "On-off-on" switch system for kanamycin determination. Reprinted with permission from Ref. [90].

Figure 5 Quantum dot fluorescent sensor for the detection of neomycin. Reprinted with permission from Ref. [95]

Figure 6 Design, fabrication, and work mechanism of MOF-based enzyme-linked immunosorbent assay. Reprinted with permission from Ref. [103]
Biotin-streptavidin reaction mediated signal amplification strategy

Biotin, also known as vitamin H, vitamin B7, or coenzyme R, has high water solubility. Streptomyces avidin can bind with biotin to form stable complexes with high specificity. Streptomycyes avidin has four subunits and can bind to four biotin molecules. Therefore, the biotin-streptavidin system is often used for signal amplification. The signal amplification system based on biotin-streptavidin has the following advantages: (i) Protein or nucleic acid molecules can be modified with biotin or streptavidin, respectively. Since one streptavidin can bind four biotins, while one protein or nucleic acid molecule can bind multiple biotins, it can achieve a multi-level amplification effect. (ii) The binding ability of biotin to streptavidin is strong, 105 to 106 times that of antigen-antibody affinity. This strong alliance can form stable complexes in an extremely short time. (iii) Various types of molecules, such as proteins, enzymes, DNA, and antibodies, have good biocompatibility with biotin or streptavidin, and their biological activity remains unchanged after modification. Therefore, according to the specific needs of detection, different biomolecules can be modified with biotin or streptavidin to achieve corresponding signal amplification effects.

Fluorescence polarization sensor based on polymerase chain reaction and biotin-streptavidin has been developed for the detection of chloramphenicol residues in dairy products. The biotin-streptavidin interaction increased the molecular weight considerably. As a result, a double-amplified fluorescence polarization signal was obtained. Under optimal conditions, it could realize a wide linear detection range of 0.001 nmol/L-200 nmol/L to develop rapid, sensitive, and simple immunosensor for the detection of kanamycin in milk [119]. This immunosensor is based on a magnetic relaxation switch assay and a
biotin-streptavidin system. The target analyte competes with the analyte on the surface of superparamagnetic iron oxide nanoparticles, thus affecting the formation of superparamagnetic iron oxide nanoparticle aggregates. The dispersed and aggregated states of superparamagnetic iron oxide nanoparticles modulate the spin-spin relaxation time of neighboring water molecules. The working range of the assay was 1.5 ng/mL-25.2 ng/mL and the LOD was 0.1 ng/mL. Compared with the ELISA (6-8 h), the LOD of this method was reduced by 10-fold, and the analysis time (45 min) was greatly shortened [120].

Conclusion

Among other detection technologies, biosensors are emerging sensing tools, especially due to the revolutionary developments in the fields of materials science and nanotechnology. However, inescapable problems associated with these sensing systems are significant barriers to the commercialization of these sensing technologies, such as low sensitivity and bio-selectivity. To address these issues, several efforts have been made to utilize nanomaterials (e.g., metallic nanoparticles, nanowires, nanosphets, quantum dots, nanochannels, graphene, and MOF) as stable signal probes for biosensors. For example, modification of electrodes by gold nanoparticles allows for electron transfer between redox-active proteins and transduction materials without the use of any mediator, thereby improving stability and sensitivity. Similarly, carbon-based (carbon nanotubes and graphene) electrodes have several advantages, which include economy, low background current, wide operating potential, and miniaturization capability. MOF-based biosensors have also been proposed for the detection of antibiotics because of their higher specificity, stability, and lower LODs compared to metal nanoparticles, quantum dots, and graphene biosensors.

The most important properties of a biosensor include specificity and sensitivity depend on the bioreceptor, biomolecule immobilization, and transduction method. The bioreceptors (such as enzymes, antibodies, DNA, and aptamers) provide specificity against the target in the developed biosensor. Another important parameter of bioreceptor sensitivity is the immobilization of biomolecules on the electrode surface without affecting their biological activity. Such an improvement can increase the stability and overall lifetime of the biosensor. However, they may still also face problems associated with converting them into portable detection systems. Future work on biosensors could focus on fully integrated and automated detection systems (such as machine-learning, and lab-on-a-chip technology) to meet the requirements of field and point-of-care applications in a practical manner.

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