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Recent developments in detection and therapeutic approaches for antibiotic-resistant bacterial infections

Recent developments in detection and therapeutic approaches for antibiotic-resistant bacterial infections

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Abstract

Owing to the widespread emergence and proliferation of antibiotic-resistant bacteria, the therapeutic benefits of antibiotics have been reduced. In addition, the ongoing evolution of multidrug-resistant pathogens poses a challenge for the scientific community to develop sensitive analytical methods and innovative antimicrobial agents for the detection and treatment of drug-resistant bacterial infections. In this review, we have described the antibiotic resistance mechanisms that occur in bacteria and summarized the recent developments in detection strategies for monitoring drug resistance using different diagnostic methods in three aspects, including electrostatic attraction, chemical reaction, and probe-free analysis. Additionally, to understand the effective inhibition of drug-resistant bacterial growth by recent nano-antibiotics, the underlying antimicrobial mechanisms and efficacy of biogenic silver nanoparticles and antimicrobial peptides, which have shown promise, and the rationale, design, and potential improvements to these methods are also highlighted in this review. Finally, the primary challenges and future trends in the rational design of facile sensing platforms and novel antibacterial agents against superbugs are discussed.

Keywords: Antibiotic-resistant bacteria, Antimicrobial peptides, Biogenic silver nanoparticles, Detection methods

1. Introduction

The discovery and clinical use of antibiotics in the field of medicine is the greatest breakthrough in the golden era of medicine in the 20th century [1]. Numerous antibiotics have been used to treat bacterial infections and have become essential clinical interventions required for the development of modern medical procedures, such as cutting-edge surgical approaches, organ transplantation, and cancer treatment [2]. Prophylactic antibiotics are prescribed in the preoperative phase for almost all surgical procedures and solid organ transplants to reduce the risk of surgical site infection [3]. Several antibiotics, including salinomycin, ciprofloxacin, and mitoxantrone, have been widely used in the treatment of cancer due to their anti-proliferative, pro-apoptotic, and anti-epithelial-mesenchymal-transition abilities [4]. Generally, antibiotics can be bacteriostatic or bactericidal [5]. Bacteriostatic agents, such as chloramphenicol, prevent bacterial cells from developing and growing, whereas bactericidal agents, such as penicillin, can directly kill bacteria [6]. Antibiotics such as macrolides, chloramphenicol, tetracycline, linezolid, and aminoglycosides target the protein synthetic machinery via interaction with ribosomal subunits, whereas

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others, such as β-lactam and glycopeptides, target the cell walls or membranes. Other groups of fluoroquinolones and rifampicin include molecules that interfere with nucleic acid synthesis, while sulfonamides and folic acid analogs act by interfering with metabolic pathways or disrupting the bacterial membrane structure [6,7].

The identification of novel antibiotics to treat infections caused by ESKAPE pathogens, such as *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter* spp., are necessary, as these Gram-negative bacteria leading to antimicrobial drug-resistant diseases on the human host is a serious threat [8]. Unfortunately, excessive consumption of antibiotics in human and animal medicine has resulted in the rise of drug-resistant bacteria worldwide, leading to global health threats [9]. Sustained consumption and improper usage of antibiotics can lead to antimicrobial resistance due to over-exposure to various drugs. Repeated exposure to small doses of antibiotics in food animals can lead to antimicrobial resistance since the larger doses of the same antibiotics have been used in human therapeutic practices. The antibiotic-resistant bacteria that food animals contain can spread to humans through the consumption of meat and poultry; or by coming in contact with animal feces in the environment [10]. Inappropriate antibiotic usage has led to the development of new resistance mechanisms and the global spread of resistant organisms. Bacterial resistance to antibiotics is a rapidly expanding problem with potentially disastrous effects and is more serious than infectious diseases caused by viruses and parasites. The rise of multidrug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE) are a major threat to healthcare and pose great difficulties for practitioners [11]. From a one health perspective, global drivers of antimicrobial resistance are complex, interdependent, and interconnected through international trade and travel [12].

To identify drug-resistant bacteria, several detection methods have been developed, including mass spectroscopy, colorimetry, fluorimetry, and electrochemistry. Additionally, efforts have also been devoted to the development of effective antimicrobial agents, including nanomaterials [13] and peptide-based [14] antimicrobial agents that have attracted wide interest due to their structure/sequence-designable properties. For instance, a fluorometric approach for the identification of polymyxin-B-resistant *Escherichia coli* (*E. coli*) has been explored based on polymyxin-B-modified upconversion nanoparticles (NPs) and anti-polymyxin-B-antibody-functionalized gold yolk–shell nanoparticles [15]. Through mecA gene recognition, the electrochemical detection of MRSA was achieved with a DNA-functionalized electrode [16]. With the self-assembly of surfactin on gold nanodots, Chen et al. explored effective antimicrobial nanomaterials against MRSA [17]. Because of the interest in drug-resistant bacteria, a summary of recent reports is meaningful for junior researchers to understand the sensing and design principles of sensors and antimicrobial agents.

Despite the contribution of some excellent reviews on the origin and detection of drug resistance, summaries that describe various detection protocols and antimicrobial agents for drug-resistant bacteria are inadequate. In this review, our objective was to summarize recent advances in the development of sensitive detection approaches and effective antimicrobial agents for drug-resistant bacteria in 2016–2021. We also describe the current challenges and future prospects for the exploitation of sensitive detection methods and efficient antimicrobial agents for combating drug-resistant bacteria.

2. Antibiotic-resistance in bacteria

2.1. Discovery of antibiotic-resistant bacteria

Antibiotic resistance in bacteria was discovered in 1940, and the selective pressure of antimicrobials has made it easier for resistant clones to survive and spread. In recent decades, the increase in antibiotic resistance observed in pathogenic bacteria has become severe [8]. Multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains of ESKAPE pathogens reportedly possess several resistance mechanisms. MDR was defined as non-susceptible to at least one agent in three or more antimicrobial categories, whereas XDR as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. PDR was defined as non-susceptible to all agents in all antimicrobial categories, respectively [18]. The emergence of microorganisms resistant to practically all existing antibiotics has become a serious and growing threat to the planet [9]. For example, MRSA is resistant to the entire β-lactam class of penicillin-like drugs. Reduced access to the penicillin-binding proteins (PBP), decrease in the binding affinity of PBP, and enzymatic degradation by β-lactamases are the three mechanisms of β-lactam resistance developed by Gram-negative bacteria. The resistance of MRSA strains is caused by the production of an altered penicillin-binding

protein 2a (PBP2a) encoded by the *mecA* gene with a lower affinity [19]. The New Delhi Metallo-lactamase (NDM-1) in a few Gram-negative bacteria (most notably *E. coli* and *K. pneumoniae*) causes resistance to carbapenems which are used as the last line of defense against multidrug-resistant pathogens. The two major mechanisms that lead to carbapenem resistance are structural mutation coupled with β-lactamase production and the pathogen's ability to generate carbapenemases, which hydrolyze the carbapenem. Moreover, efflux pumps and modifications to penicillin-binding proteins are also responsible for the emergence of carbapenem resistance in bacteria [20]. Because of the emergence and spread of antibiotic resistance among microorganisms, the efficacy of antibiotic treatment is decreasing. Thus, the development of newer and more promising antibiotics capable of long-term potency against a variety of life-threatening illnesses is required [5].

Antimicrobial resistance is a long-standing phenomenon that occurs naturally because of interactions between numerous organisms and their surroundings. As the majority of antimicrobial substances are naturally occurring chemicals, coexisting bacteria have evolved methods to counteract their effects to survive. In clinical microbiology laboratories, clinical breakpoints are used to classify microorganisms as clinically susceptible (S), intermediate (I), or resistant (R), depending on the quantitative antimicrobial susceptibility as indicated by the MIC value determined in a standard test system [2]. Susceptibility breakpoints are a relatively constant metric for evaluating the efficiency of treatment and stimulate the evaluation and selection of antimicrobial regimens in a typical patient group under fixed exposure conditions [21]. When a resistant mutant forms, the antibiotic scavenges the susceptible population, leaving only resistant bacteria to survive.

The inherited ability of a bacterium to thrive when exposed to high quantities of antibiotics is called resistance which can be classified into three different types: intrinsic, phenotypic, and acquired resistance. Intrinsic resistance includes a group of factors that contribute directly or indirectly to antibiotic resistance, regardless of previous antibiotic exposure and the absence of horizontal gene transfer (HGT). The mechanism of intrinsic resistance comprises antibiotic inactivation, target modification, and changes in bacterial permeability. Phenotypic resistance is non-inheritable and occurs when a susceptible bacterial population becomes resistant for a short period. It can occur via the development of persistence, swarming adaptation, and biofilm growth. Acquired resistance takes place by mutating these elements and resistance gene acquisition through HGT. The acquired resistance mechanisms also include antibiotic inactivation, target modification, and antibiotic efflux [12].

2.2. Mechanism of antibiotic resistance in bacteria

As shown in Fig. 1, antimicrobial resistance in bacteria can be caused by three major mechanisms [12], (A) inactivation of antibiotics, (B) modification of the drug target, and (C) reduced permeability and/or increased active flux. Antimicrobial resistance can be either inherent or acquired and can arise from mutations in existing genes or the transfer of genes from other species or strains [11].

(A) Enzymatic inactivation of antibiotics

Enzymatic inactivation is a major antibiotic resistance mechanism in bacterial isolates. Drug inactivation occurs when hydrolytic enzymes from bacteria, such as β-lactamases and TetX, catalyze the oxygen-dependent destruction of tetracyclines. Transferring a chemical group to a drug, such as an acetyl, phosphoryl, or adenyl group causes drug inactivation. Different forms of aminoglycoside-modifying enzymes, which include both N-acetyl transferases and phosphotransferases, have been detected in Gram-positive and negative bacteria in cases of resistance to aminoglycosides [12].

(B) Modification of the drug target

Antibiotic targets are chemically altered by enzymes produced by bacteria, as evident by the acquired antibiotic resistance. Resistance to antibiotics, such as penicillin-binding proteins, can be induced by transpeptidases on the cell walls of bacteria. Any change in transpeptidase number or structure can affect the number of antibiotics attached to the target. Alternative anti-bacterial methods include target replacement and protection as well as changes in the target sites [12]. For example, a β-lactam-resistant bacterial strain possesses new PBP genes with altered structures. MRSA harbors the *mecA* gene, which encodes the production of PBP2a proteins to lower the binding affinity to β-lactam antibiotics.

(C) Reduced permeability and/or increased active flux

Drug efflux is one of the major mechanisms of antibiotic resistance in clinical isolates. Antibiotic

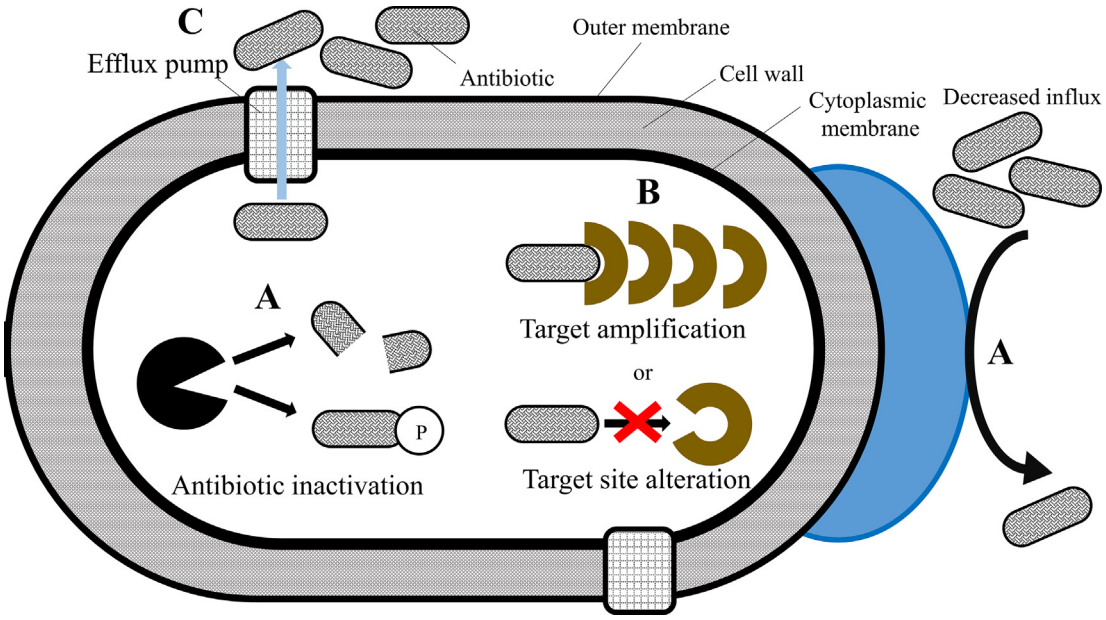

Fig. 1. Antibiotic resistance mechanism in bacteria. (A) antibiotics inactivation, (B) modification of drug target, and (C) reduced permeability and/or increased active flux.

efflux pump genes can be acquired or are inherent. In both types of bacteria, active efflux pumps are classified into five families: the ATP-binding cassette family (ABC), major facilitator superfamily (MFS), small multidrug resistance family, multidrug and toxic compound extrusion family, and resistance-nodulation-cell-division family (RND). The composition, type of substrate, energy source, and the number of transmembrane spanning regions influence the classification of efflux pumps in this family. In Gram-positive bacteria, active efflux pumps belong to the ABC family, and the microbes use ATP as an energy source and show a minor role in the transmission of MDR. The MFS superfamily, which is predominantly found in Gram-positive bacteria, and the RND family, which is found in Gram-negative bacteria, are associated with antibiotic resistance [7,12].

3. Detection of drug-resistant bacteria

As mentioned above, WHO labels drug-resistant bacteria as a serious threat, and may cause large numbers of deaths in the future. Therefore, the development of sensitive and selective methods for detecting drug-resistant bacteria is required, which will benefit the deep understanding of drug resistance in bacteria and the monitoring of environmental quality. Different drug-resistant bacteria possess various properties, including a mutation region, enzyme disorder, and a flagellum structure. The high diversity of different drug-resistant bacteria has inspired the exploration of various methods for their sensitive detection by recognition of different targets, including RNA, DNA, enzymes, and surface charges [22–24]. Accordingly, sensing systems for drug-resistant bacterial detection based on optical spectrometry, mass spectrometry, and electrochemistry have been constructed based on three aspects: electrostatic attraction, chemical reaction, and probe-free analysis.

3.1. Chemical/biochemical reaction-based detection

Genetic and metabolic abnormalities can lead to abnormal levels of various biocomponents in drug-resistant bacteria [25]. These biocomponents, including DNA, RNA, enzymes, and proteins, can act as biomarkers for identifying drug-resistant bacteria [26–28]. Thus, the detection of these biomarkers is essential for determining the presence of drug-resistant bacteria. Given this characteristic, the development of sensitive methods for detecting specific drug-resistant bacteria-related gene sequences, enzymes, and small molecules, based on chemical/biochemical reactions, has attracted growing attention in recent years.

Drug stimulation can easily cause gene mutations and transform normal bacteria into drug-resistant bacteria; thus, detection of mutated gene sequences is important for the discrimination of drug-resistant bacteria. For example, Suea-Ngam et al. developed an electrochemical approach for recognizing the

DNA target of MRSA based on a Cas12a-sgRNA complex-modified electrode [16]. The sensing mechanism is illustrated in Fig. 2A, where the addition of the target DNA induces the formation of the Cas12a-sgRNA-target complex, which activates the Cas12a enzyme and initiates the degradation of the DNA linker. The degraded DNA linker inhibits the decomposition of AgNPs. The electrochemical signal decreased as the concentration of the target DNA increased. With this design, the linear detection range of target DNA from 10 fM to 0.1 nM was realized, and the limit of detection (LOD) was determined to be 10 fM. The specificity of this tactic was evaluated by testing colonies of *E. coli, Enterococcus faecalis (E. faecalis), Listeria monocytogenes (L. monocytogenes), Staphylococcus epidermidis (S. epidermidis)*, and methicillin-sensitive *S. aureus*. Interestingly, none of these induced a comparable decrease in the electrochemical signal, indicating that this approach is selective toward MRSA.

Similarly, Zhang et al. proposed an electrochemical system for the analysis of the NDM-1 gene by integrating Au nanocages (Au NCs)/reduced graphene oxide-functionalized zinc oxide (ZnO) electrodes, and extra thiolated locked nucleic acids and Au NCs [29]. As shown in Fig. 2B, the introduction of NDM-1 DNA resulted in the formation of a sandwich structure, which increased the density of the Au NCs, adsorbed $[Fe(CN)_6]^{3-/4-}$ on the electrode surface, and boosted the electrochemical signal. Signal stability was further enhanced by constructing a 2×8 array on an electrochemical biochip. Data from all electrodes were acquired synchronously and analyzed in parallel. The system enabled the detection of NDM-1 DNA in the concentration range of 1−100 μg/L, and the LOD was 0.042 pg/L under optimal conditions. In view of the high sensitivity of fluorescence techniques, fluorimetric analysis of DNA targets, reactive species, and ions are reported with proper probes [30]. With the combination of CRISPR/Cas9 excision and plasmid cycle, Goyal et al. presented a fluorimetric β-lactamase DNA detection by single-molecule fluorescence microscopy [31]. In this work, the Cas9 recognized the target gene and induced the cleavage of a double-stranded DNA, causing the circular plasmid to linearize. The circular to a linear configuration change of plasmid appeared and could be visualized with fluorescence microscopy quickly. They also achieved the determination of plasmids number and sizes in a sample with the proposed system.

To further enhance the accuracy of the gene detection, multiple discriminant analysis was performed. Using electrochemical and mass spectral immunoassays, Pugia et al. developed the culture-free detection of the β-lactamase and cefotaximase resistance genes [32]. Based on this strategy, drug-resistant gene sequences were detected using both electrochemical and mass spectral immunoassays, resulting in highly accurate detection. However, samples with a negative response to rapid electrochemical immunoassays could not be confirmed by mass spectral immunoassays. The total number of antimicrobial resistance genes was verified by quantitative polymerase chain reaction (qPCR).

In addition to the identification of a single gene target, multiplex and high-throughput DNA sequencing are important for detecting drug-resistant bacterial communities and predicting antimicrobial resistance. Genomic analysis facilitates the recognition of various gene sequences within a short timeframe. For example, gene pollution in farmland soil after long-term fertilization with chicken manure in Shandong Province was determined using this strategy [33]. The chickens were treated with tetracycline or sulfonamide antibiotics before fertilization for fecaluria, and gene sequencing of 11 potential targets was performed using qPCR analysis. Zhao et al. found that the resistance genes *tetW* and *tetO* appeared with the highest frequency after tetracycline antibiotic treatment, whereas *sul1* and *sul2* showed a high probability with sulfonamide antibiotic treatment. Fang et al. tested the bacterial communities and diversity of antibiotic resistance genes in *Eriocheir sinensis* aquaculture ponds using high-throughput sequencing-based metagenomic approaches. The bacterial communities differed among the various samples. The dominant phyla in water samples were *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*, and *Proteobacteria*, *Chloroflexi*, *Verrucomicrobia*, and *Bacteroidetes* were the predominant phyla in sediment samples. The group also found that plasmids were the most abundant mobile genetic elements and strongly correlated with antibiotic resistance genes. Their results suggest that opportunistic pathogens may become drug-resistant through HGT and increase the potential risk to human health.

Regarding widespread drug-resistant bacteria, the causal relationships between bacteria, genes, and the corresponding association with antibiotic use need to be well understood. Cai et al. investigated bacterial-specific DNA sequences in hospital sewage and performed network analysis to predict clinical antimicrobial resistance [28]. Based on their data, 1573 bacterial species and 885 drug-resistant genes were detected in sewage. The results showed that different antibiotics possessed diverse

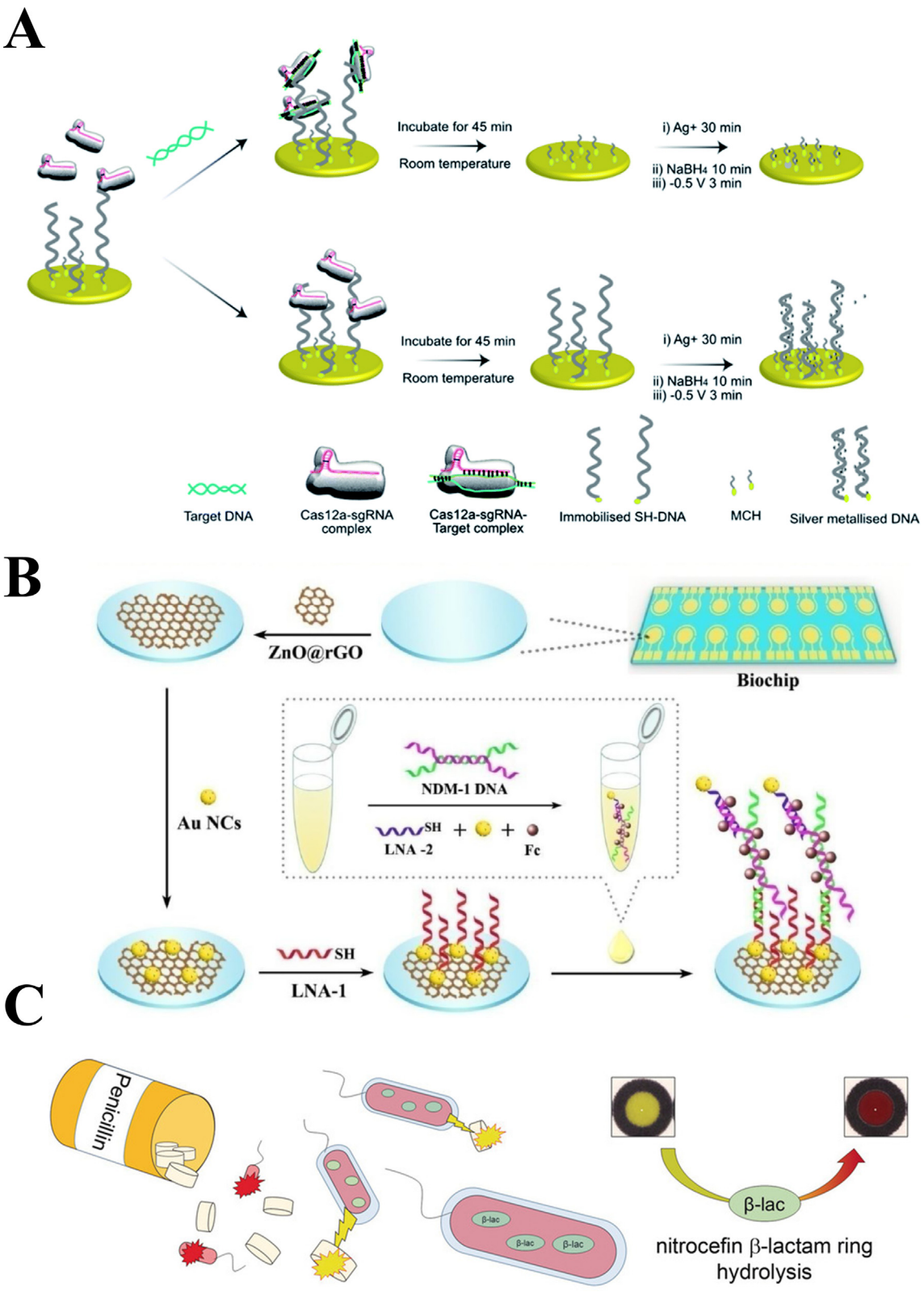

Fig. 2. (A) Schematic illustration of the E-Si-CRISPR technique. Reprinted from [16] with permission from the Royal Society of Chemistry, Copyright 2021. (B) Schematic representation of fabrication of the Au NC@LNA-1/NDM-1DNA/LNA-2@Au NC "sandwich-like" complex modified 2 × 8 array electrochemical biochips. Reprinted from [29] with permission from Elsevier, Copyright 2020. (C) Scheme of the paper device-based fast antimicrobial-resistant bacteria detection assay by the presence of β-lactamase-mediated. Reprinted from [34] with permission from Wiley, Copyright 2017.

resistance inducibility to aminoglycosides, sulfonamides, tetracyclines, phenicols, macrolides, and quinolones. Additionally, the co-occurrence patterns reflect the positive or negative correlations between drug-resistant bacteria as well as genes and can help predict drug-induced resistance.

Monitoring enzyme abnormalities is also an effective means for the identification of drug-resistant bacteria. The commonly investigated enzyme is β-lactamase; its overexpression in several β-lactam antibiotic-treated bacteria inactivates β-lactams by hydrolyzing the β-lactam ring. Because of the high sensitivity and easy operation, optical spectrometry has been extensively applied in drug-resistant bacteria detection. As an example, Boehle et al. developed a paper-based analytical device based on β-lactamase-catalyzed hydrolysis of the carbon–nitrogen bond in nitrocefin and analyzed the subsequent color change (Fig. 2C) [34]. Using *E. coli* as the demonstration bacteria, β-lactamase-expressing *E. coli* alone caused distinct color changes, whereas control *E. coli* did not have a similar effect. However, the macroscopic color change requires a high concentration of hydrolyzed nitrocefin; this assay works when the bacterial concentration is greater than 3.8×10^6 CFU/mL. Gelatinase is also an important enzyme in drug-resistant bacteria and can act as a biomarker for MRSA. Lin et al. explored a nanosystem consisting of Ru-complex-functionalized Se nanoparticles, gelatin nanoparticles, and natural red blood cell membranes and denoted it as Ru–Se@GNP-RBCM. Gelatinase-mediated hydrolysis of gelatin nanoparticles resulted in the release of fluorescent Ru-complex-functionalized Se nanoparticles, which acted as fluorescence reporters for MRSA. The natural red blood cell membrane reduces immune system clearance and enables direct and effective MRSA imaging. In addition, Du et al. established a sensitive and fluorimetric diagnosis method for MRSA based on MRSA-identifiable aptamer and gelatinase-responsive heptapeptide linker–cypate complexes cofunctionalized gold nanostars, denoted as AuNS–Apt–Cy nanoprobe [35]. The gelatinase-mediated fluorescence increment and aptamer-targeted characters allowed specific recognition of 10^5 CFU MRSA. The high fluorescence signal in MRSA-infected wounds of diabetic mice also demonstrated the practical *in vivo* application of the proposed AuNS–Apt–Cy nanoprobe.

3.2. Electrostatic attraction-based detection

The bacterial cell membrane is composed of a bilayer of phospholipids (liposomes), whereas the flagellum surface is composed of a layer of lipopolysaccharide (LPS), making the surface of the bacteria negatively charged. Therefore, positively charged materials are easily adsorbed onto the surface of bacteria via electrostatic attraction, and further endocytosis leads to their uptake. Thus, several positively charged materials have been explored as fluorescence probes for the detection or imaging of various drug-resistant bacteria. Unlike chemical-reaction-based sensing, electrostatic attraction does not require a specific interaction other than a positive charge. However, some environment-dependent charge states may cause false-positive results. To enable the effective and reliable detection and imaging of drug-resistant bacteria, probes with stable positive charges have been developed by introducing a metal complex, quaternary ammonium, and triphenylphosphine.

Notably, electrostatic attraction-mediated recognition usually results in probe aggregation. However, conventional fluorophores display aggregation-caused quenching. That is, these fluorophores show weak fluorescence at high molar concentration or solid state due to the small Stokes shift and strong self-absorption [36,37]. Compared to these traditional fluorophores, a new type of emitter with aggregation-induced/enhanced emission called aggregation-induced emission fluorogens (AIEgens), has attracted interest in sensing and imaging applications [38]. To overcome the drawbacks of traditional fluorophores, AIEgen-anchored positively charged probes have been explored. For example, cyclometalated iridium(III) complexes with aggregation-induced emission characteristics have been used for rapid and sensitive detection of carbapenem-resistant *A. baumannii* and MRSA [39]. The sensing mechanism was attributed to the aggregation and fluorescence increase caused due to iridium(III) complexes induced by bacteria-released endotoxins, including LPS and lipoteichoic acid (LTA). This approach enabled bacterial detection with the naked eye at 10^8 CFU/mL and endowed sensitive detection at 10 CFU/mL by integrating fluorimetric spectroscopy. Similarly, Usman et al. demonstrated the utility of ruthenium arene complexes for drug-resistant gene detection owing to their high binding affinity [40]. The fluorescence emission of the ruthenium arene complexes decreased with increasing target DNA concentration without changing its profile. Through metal-DNA interactions, the ruthenium complexes detected pathogens including *P. aeruginosa* PAO1, *Chromobacterium violaceum* (ATCC 12472), and MRSA.

Quaternary ammonium and triphenylphosphine are the strongest positive groups besides metal complexes and have been commonly used for

organelle localization. A few studies have reported the development of quaternary ammonium- and triphenylphosphine-linked probes for detecting drug-resistant bacteria. By exploring tetraphenylethylene as the fluorophore and pyridinium as the responsive motif, Xie et al. developed a 1,2-Diphenyl-1,2-bis(4-(2-(pyridine-4-yl)vinyl)phenyl) ethene probe (denoted as DBPVE) for detecting drug-resistant bacteria [41]. As shown in Fig. 3A, The two pyridinium groups with different hydrophobicities in the DBPVE probe enabled rapid and effective bacterial membrane binding. Using methicillin-resistant *S. epidermidis* as the model bacteria, strong fluorescence was observed after incubation with the DBPVE probe. The length of the alkyl chain also affects the binding affinity towards methicillin-resistant *S. epidermidis*.

Drug-induced resistance in some bacteria changes the phosphate group of LPS into amine-terminated phosphate, which reduces the negative surface charge of bacteria. Utilizing this property, Sun et al. proposed a competitive system for detecting polymyxin B-resistant *E. coli*. based on polymyxin-B-linked upconversion nanoparticles (UCNPs) and polymyxin-B antibody-modified gold yolk−shell nanoparticles (Au YS) [15]. The positively charged polymyxin-B drug can easily bind to polymyxin-B-sensitive *E. coli* with LPS on the cell surface, which inhibits the subsequent connection of gold yolk−shell nanoparticles via antigen−antibody interactions. As a result, the fluorescence of the UCNPs was not reduced. In contrast, phosphate mutation in LPS of polymyxin B-resistant *E. coli* restricts the approach of UCNPs and facilitates coupling between UCNPs and gold yolk−shell nanoparticles, leading to dramatic fluorescence suppression, as shown in Fig. 3B. In addition, the coupling also causes an increase in the circular dichroism signal of the gold yolk−shell nanoparticles modified by the polymyxin-B antibody. Therefore, these two signals can be used to determine the concentration of polymyxin B-resistant *E. coli*.

The above methods detect one or two specific drug-resistant bacteria alone, whereas the diversity of bacteria suggests the strong potential of multiple bacterial analyses. Array-based sensing tactics are alternative candidates for multi-target analysis. This strategy is commonly called the "chemical nose" technique, and can obtain simultaneous discrimination of various analytes in two steps: (1) generation of a unique response pattern for a certain target by selective rather than specific interaction between analyte-sensing elements, and (2) digitization and grouping of various response patterns through linear discriminant analysis (LDA). With the use of array-based sensing approaches, high-throughput analysis of multiple targets, including proteins, isomers, and analogs, has been reported [42−44]. Using this technique, Yang et al. constructed a gold nanoparticle@gold nanocluster nanocomposite-based fluorescence sensor array for the discrimination of drug-resistant bacteria [45]. In this system, negatively charged vancomycin-templated gold nanoclusters (Van-AuNCs) were first fixed onto positively charged cetyltrimethylammonium bromide (CTAB)-capped gold nanoparticle (CTAB-AuNPs) surfaces by electrostatic attraction, which endows nanocomposites with visible absorbance, fluorescence, and scattering. However, the strong binding affinity between CTAB and the negatively charged bacteria led to the liberation of Van-AuNCs, which disassembled the nanocomposite, induced a decrease in absorbance and scattering, and an increase in fluorescence. The variation in the signals of the three channels enables the generation of a unique pattern for specific bacteria. Through LDA, they incorporated different drug-resistant Gram-negative bacteria, including *E. coli* O157:H7, *P. aeruginosa*, and *P. vulgaris*. Additionally, this system also enabled the differentiation of two strains of non-resistant *E. coli* and three common pathogenic bacteria, *Y. mollaretii*, *P. putida*, and *P. vulgaris* from three drug-resistant bacteria. On the basis of electrostatic competition, Behera et al. reported a fluorimetric method to discriminate the bacterial drug resistivity by cationic two-dimensional MoS_2 units and green fluorescence protein [46]. The electrostatic attraction between MoS_2 units and green fluorescence protein led to surface adsorption and fluorescence quenching. However, the bacterial binding released green fluorescence protein and recovered the fluorescence. With the use of LDA technique, they realized the differentiation of six different bacterial strains.

3.3. Probe-free analysis

Despite the exploration of sensitive detection and imaging of drug-resistant bacteria, the above-mentioned methods require the use of specific/selective probes. Recently, probe-free detection approaches have attracted growing research interest owing to their real-time and on-site analysis characteristics. Unlike chemical reactions or electrostatic attraction-mediated assays, probe-free analysis emphasizes the self-response of the target molecules with special stimulation. Few techniques, including mass spectroscopy and vibrational spectroscopy, are applicable for probe-free drug-resistant bacterial analysis.

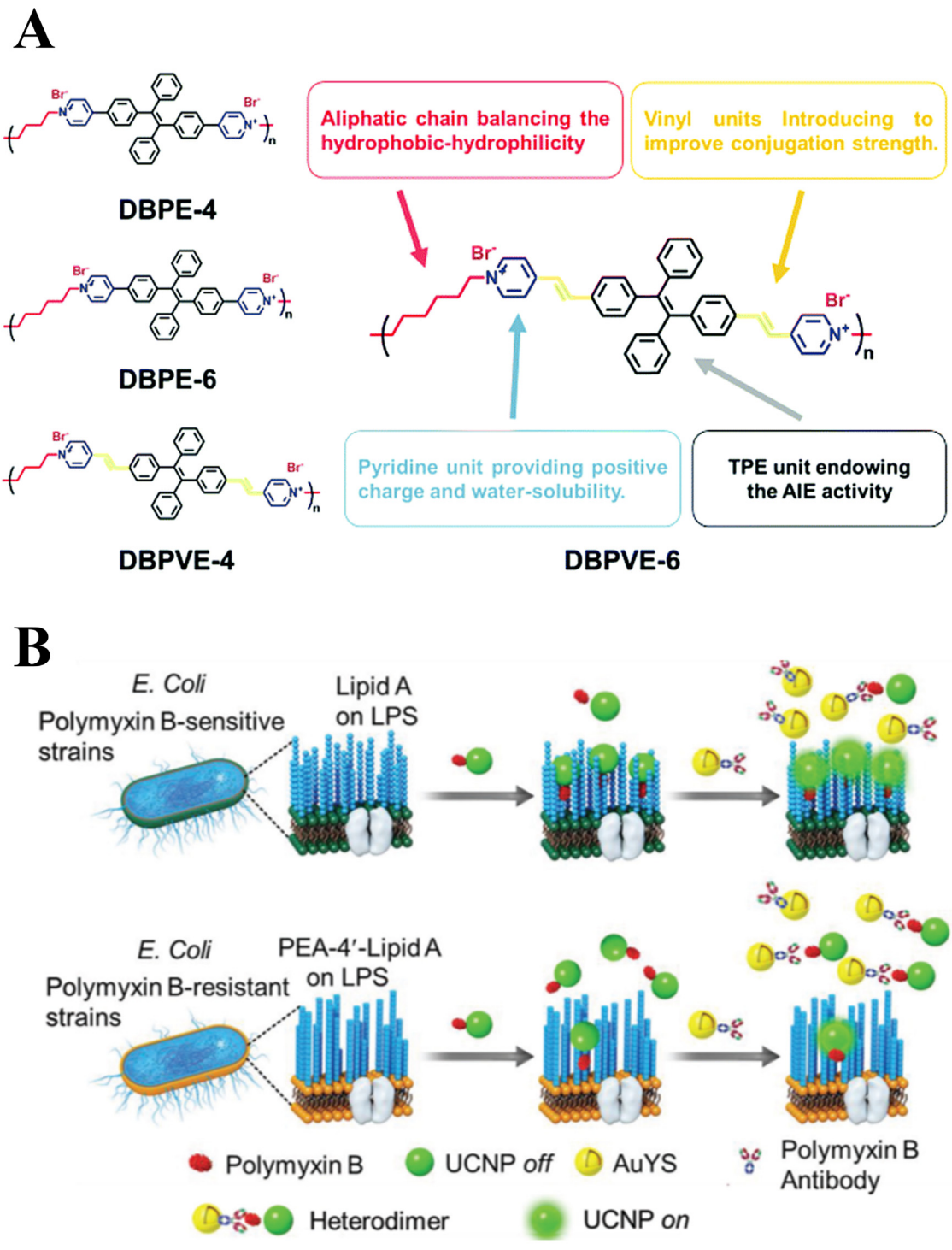

Fig. 3. (A) Chemical structures and function explanation of AIE-active DBPVE probes for antibiotic-resistant bacterial sensing. Reprinted from [41] with permission from the Royal Society of Chemistry, Copyright 2021. (B) Schematic illustration of Au YS and UCNP heterodimer for quantitative detection and imaging of drug-resistant bacteria. Reprinted from [15] with permission from Wiley, Copyright 2018.

Mass spectroscopy identifies various targets directly and has been widely used for diagnosis and detection [47,48]. For example, using solid-phase extraction, Mokh et al. reported the implementation of liquid chromatography coupled to tandem mass spectrometry (SPE-LC-MS) for the simultaneous determination of 63 pharmaceuticals (*i.e.*, antibiotics, stimulants, antidepressants, mucolytics, and

antiparasitic agents) and some metabolites [49]. The mass signals from the pharmaceuticals and metabolites were collected and aligned with those of drug-resistant bacteria. For various species, the LODs ranged from 2.3 to 94.3 ng/L. In addition to the simple detection of bacteria, drug resistance can be evaluated using this method. The results showed that intestinal *Enterococcus* and *E. coli* can easily become resistant strains upon treatment with various antibiotics, whereas *P. aeruginosa* was resistant to only one antibiotic studied. Using a sandwich microfluidic filter device, Zhang et al. proposed an online electrospray ionization mass spectrometry (ESI-MS) technique for drug resistance analysis [50]. The bacteria were first blocked on the microfluidic chip, and subsequently, stimulation was provided by injecting beta-lactam antibiotic drugs. The antibiotics and related hydrolysis products were detected by online ESI-MS analysis, which enabled the assessment of drug resistance within 30 min. Based on this approach, the authors discriminated against four *E. coli* strains, with two resistant and two non-resistant strains through stimulation with ampicillin and third-generation cephalosporin ceftriaxone.

Besides the detected pharmaceuticals and metabolites/hydrolysis products, drug-induced resistance changes the composition of bacterial cell membranes, making cell membrane analysis important for identifying drug-resistant bacteria. Based on microbial membrane lipid fingerprinting, Liang et al. identified clinically relevant Gram-positive and Gram-negative pathogens using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [51]. In the study, lipids were efficiently extracted from microbial membranes or fungal species using an aqueous sodium acetate buffer for 1 h. Pearson's correlation analysis revealed that the lipid fingerprints were associated with the resistance characteristics of various bacteria. To facilitate the understanding of bacterial detection, partial detection methods are summarized in Table 1.

4. Inhibition of antibiotic-resistant bacterial growth using nanomaterials

Nanomaterials are beneficial for antibacterial therapies due to their distinct properties as compared to their bulk form. Additionally, they provide benefits such as antibacterial drug delivery systems, resisting biofilm, and drug-resistant mutants [52,53]. Nanomaterials can be further categorized as carbon allotrope-based, inorganic, organic, and composite-based nanomaterials based on their physical and chemical properties [54]. Antibacterial nanomaterials have a variety of mechanisms of action, which is a key benefit because it makes it difficult for bacteria to adapt and acquire resistance [55]. Metal NPs have demonstrated good antibacterial activity in a large number of trials and are among the most promising emerging antibiotic agents [13]. The generation of reactive oxygen species (ROS) and membrane disruption was attributed to the toxic mechanism of action for metal NPs against bacterial cells. Additionally, a variety of nanomaterials, such as lipid nanoparticles, nanoclusters, carbon dots, chitosan, polymeric nanoparticles, fullerenes, carbon nanotubes, and nanocomposites also exhibit antibacterial action [56–60]. The bactericidal action of NPs is influenced by several parameters, including size, stability, surface area, and mechanical strength. These characteristics make them ideal for the treatment of microbial infections [61]. Several metal-based nanoparticles, such as silver (Ag), copper, gold, molybdenum disulfide, and metal oxides such as copper oxide, ZnO, cerium oxide, titanium dioxide, aluminum oxide, magnesium oxide, and magnetic

Table 1. Summary of drug-resistant bacteria detection based on various strategies.

Strategy	Drug-resistant bacteria	Target	LOD	Ref
Biochemical reaction	MRSA[a]	*MecA* gene	3.5 fM DNA	[16]
Biochemical reaction	MRSA[a]	16S rRNA gene	100 cells	[32]
Biochemical reaction	[b]	NDM-1 gene	0.042 pg/L DNA	[29]
Biochemical reaction	β-*Lactam antibiotics-resistant E. coli*	β-lactamase	3.8×10^6 CFU/mL	[34]
Electrostatic attraction	*Polymyxin-B-resistant E. coli*	Lipoteichoic acid	1×10^3 CFU/mL	[15]
Electrostatic attraction	MRSA[a]	Lipoteichoic acid	1.2 CFU/mL	[39]
Probe-free (mass spectroscopy)	*Carbapenem-resistant E. coli*	Resistance protein	[b]	[88]
Probe-free (mass spectroscopy)	MRSA[a]	Membrane lipids	1.0×10^5 CFU/mL	[51]
Probe-free (infrared spectroscopy)	*Antibiotics-resistant E. coli*	Lipid/Protein	[b]	[89]
Probe-free (Raman spectroscopy)	MRSA[a]	Serogroups	[b]	[90]
Probe-free (Vibrational spectroscopy)	MRSA[a]	oxacillin-induced chemical changes	[b]	[91]

[a] MRSA: Methicillin-resistant *Staphylococcus aureus*.
[b] Not available.

Table 2. Biogenic AgNPs synthesized using different parts of plants against MDR bacteria.

Plant/Derivative	Size (nm)	Target MDR pathogens	MIC (μg mL^{-1})	Ref
Astragalus membranaceus roots	65.08	MRSA[b]	63	[92]
		MRSE[d]	63	
		E. coli[a]	32	
		P. aeruginosa[a]	32	
Ocimum gratissimum leaves	16 ± 2	*E. coli*[a]	4	[93]
		S. aureus[a]	8	
Phyllanthus amarus whole plant	24 ± 8	*P. aeruginosa*[a]	6.25–12.5	[94]
Acacia rigidula stems and roots	22.46 ± 10.83	*P. aeruginosa*[a]	7.8	[95]
Origanum majorana leaves	26.63	MRSA[b]	20	[96]
		A. baumannii[a]	40	
		K. pneumonia[a]	10	
Convolvulus fruticosus aerial parts	45	*S. aureus*[a]	17	[71]
		E. faecalis[a]	1	
		A. baumannii[a]	4	
		E. coli[a]	4	
		P. mirabilis[a]	2	
		K. pneumonia[a]	2	
		P. aeruginosa[a]	2	
Solanum xanthocarpum fruit	22.45	*E. coli*[a]	2500	[97]
		Shigella sp.[a]	2500	
		P. aeruginosa[a]	1250	
		Aeromonas sp.[a]	1250	
Juniperus excels leaves	16.08–24.42	MRSA[b]	48 ± 2.02	[98]
		MSSA[c]	48 ± 1.15	
Areca catechu fruits	25	VRE[e]	11.25	[70]
		P. aeruginosa[a]	5.6	
		A. baumannii[a]	5.6	
Aloe vera leaves	30–80	*E. coli*[a]	50	[99]
		A. baumanii[a]	50	
		P. aeruginosa[a]	50	
		S. aureus[a]	100	
Momordica charantia fruits	16.4 ± 4.9	*A. baumannii* (CR32X 17978)[g]	4	[72]
		A. baumannii (IMP32X 17978)[h]	4	
Stachys inflata leaves	35–45	*S. aureus*[a]	7	[100]
		P. aeruginosa[a]	0.9	
		E. faecalis[a]	0.2	
		K. pneumonia[a]	0.2	
		E. coli[a]	0.4	
		P. mirabilis[a]	0.9	
		A. baumannii[a]	0.4	
Murraya koenigii leaves	5–20	MRSA[b]	32	[101]
		MSSA[c]	32	
		E. coli (EsβL)[i]	64	
Helicteres isora fruits	8–20	XDRPA[f]	300	[102]
Caesalpinia sappan heart wood	30.2–47.5	MRSA[b]	75–150	[103]
Tinospora Cordifolia stem	9 ± 36	*P. aeruginosa*[a] (20 strains)	6.25–200	[104]
Echinochloa stagnina shoots	30	*K. oxytoca*[a]	12.5	[105]
		P. aeruginosa[a]	6.5	
Cinnamomum tamala leaves	10–12	*E. coli*[a]	12.5	[106]
		K. pneumonia[a]	10	
		S. aureus[a]	12.5	
Sambucus ebulus several parts	35–50	*S. aureus*[a]	25	[107]
		E. faecalis[a]	100	
		P. aeruginosa[a]	1.5	
		A. baumannii[a]	3.125	
		E. coli[a]	3.125	
		K. pneumoniae[a]	50	
		P. mirabilis[a]	6.25	

(*continued on next page*)

Table 2. (continued)

Plant/Derivative	Size (nm)	Target MDR pathogens	MIC (μg mL^{-1})	Ref
Rubella tuberosa leaves	55.65	*E. aerogene*[a]	16	[108]
		S. aureus[a]	8	
		K. pneumoniae[a]	32	
		A. baumannii[a]	16	
		P. aeruginosa[a]	4	
		E. faecium[a]	>128	
		S. typhi[a]	>16	

[a] Multidrug-resistant bacteria.
[b] MRSA: Methicillin-resistant *Staphylococcus aureus*.
[c] MSSA: Methicillin-susceptible *Staphylococcus aureus*.
[d] MRSE: Methicillin-resistant *Staphylococcus epidermidis*.
[e] VRE: Vancomycin-resistant *Enterococcus faecalis*.
[f] XDRPA: Extensively drug resistant *Pseudomonas aeruginosa*.
[g] CR32X 17978: colistin-resistant *Acinetobacter baumannii*.
[h] IMP32X 17978: imipenem-resistant *Acinetobacter baumannii*.
[i] EsβL: extended-spectrum β-lactamase.

iron oxide, have received considerable attention because of their impact on human health, including antimicrobial applications [62–65].

Silver is known to have intensive antibacterial properties since ancient times [66]. Compared to other metals in their nano form, nanoscale silver particles with a high surface-area-to-volume ratio (i.e., size below 100 nm) are of particular interest due to their high antibacterial effects against bacteria, viruses, and other eukaryotic pathogens. The surface area of AgNPs is one of the most critical elements for their antimicrobial properties, and AgNPs can continuously release Ag^+ ions into and out of bacteria. The maximum concentration of released Ag^+ was observed in AgNPs with the largest surface area. AgNPs were shown to be highly effective against bacteria with resistant pathogenic activity, causing cell damage due to the release of silver ions from the nanoparticles [67].

Owing to the growing popularity of green approaches, NPs are now being synthesized from a variety of green sources, including bacteria, fungi, algae, and plant extracts. The use of green synthesis to produce biogenic NPs has several advantages, including the production of stable NPs, providing an additional active surface area for interaction in the biological environment, the absence of hazardous byproducts, and cost-effectiveness [68]. Biological molecules from the sources mentioned above can generate NPs and cover NP surfaces to improve their antimicrobial activity. The green synthesis of biogenic AgNPs as antibacterial agents using different plant systems is summarized in Table 2. There are three processes for the bioreduction of metal NPs using plant extracts. The first is the activation stage, which involves the reduction and nucleation of metal ions. Second, the small adjoining NPs merge to form larger particles, which is accompanied by an increase in the thermodynamic stability of the NPs, referred to as the growth phase. Finally, the termination phase determines the shape of the NPs [69].

To understand antibacterial performance against drug-resistant bacteria, Choi et al. investigated the antibacterial activity of AgNPs synthesized using *Areca catechu* extracts against three antibiotic-susceptible and three antibiotic-resistant bacteria. After incubation with biogenic AgNPs for 24 h, the minimum inhibitory concentration (MIC) against vancomycin-resistant *E. faecalis* was 11.25 μg/mL, whereas that against multidrug-resistant *P. aeruginosa* and multidrug-resistant *A. baumannii* was μg/mL, with a MIC value of 5.6 μg/mL [70].

Using *Convolvulus fruticosus* extracts as a reducing agent, Ahodashti et al. [71] reported the synthesis of AgNPs with efficient antibacterial activity against multidrug-resistant strains of *S. aureus, E. faecalis, A. baumannii, E. coli, P. mirabilis, Klebsiella pneumonia*, and *P. aeruginosa* with MIC values of 17,1, 4, 4, 2, 2, and 2 μg/mL, respectively. Kavya et al. reported the systematic evaluation of the antioxidant efficiency and antibacterial mechanism of bitter gourd extract-stabilized AgNPs. The combination of plant extracts and AgNPs induced a synergistic effect that enhanced the antimicrobial effectiveness of plant extract-mediated AgNPs against *E.coli, P. aeruginosa, A. baumannii, S. aureus*, and colistin- and imipenem-resistant *A.baumannii.* Evaluation of the antibacterial mechanism of biogenic AgNPs indicated that the accumulation of AgNPs on the bacterial cell surface, steady release of silver ions, and production of ROS led to irreversible structural damage to bacterial cells [72].

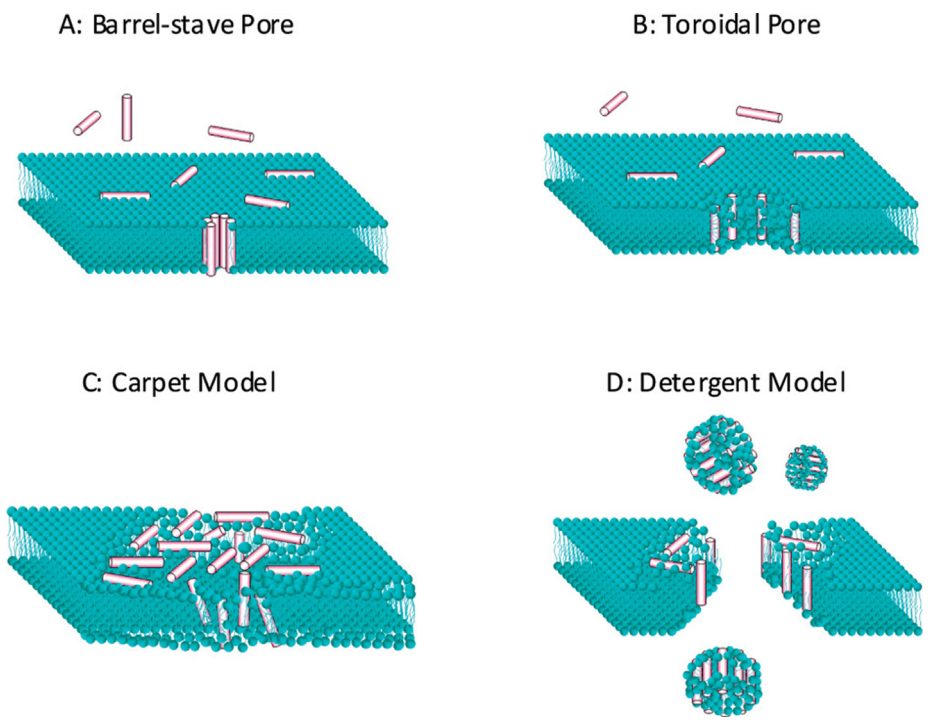

Fig. 4. Commonly cited models for antimicrobial peptide activity. Reprinted from [79] with permission from the ACS Chemical Biology, Copyright 2010.

5. Inhibition of antibiotic-resistant bacterial growth using antimicrobial peptides

Antimicrobial peptides (AMPs) are a promising class of potential drug candidates in the era of multidrug resistance to combat communicable and non-communicable diseases [14,73]. AMPs have been termed "natural antibiotics" because they can activate the innate immune response and combat a wide variety of pathogens [74,75]. AMPs are abundantly expressed and promptly activated at epithelial surfaces to combat invasion by a variety of pathogens. The innate immune pathway is activated by AMPs without any memory, by providing shorter, non-specific wide spectrum protection to the host. Several cellular functions, such as cytokine release, chemotaxis, antigen presentation, angiogenesis, and wound healing, are influenced by various AMPs thereby altering the characteristics of the mammalian membrane or by interacting with its receptors. The resolution of infection and repair of injured epithelia are aided by these functions, which complement their antibacterial effect [76]. Structural characteristics include net charge (i.e., cationic or anionic), structural conformation (i.e., α-helical, linear/extended, β-sheet, and cyclic), amphipathic properties, and an amino acid-based sequence, which affect the antibacterial activity of AMPs against microorganisms [14,77]. In general, AMPs contain 5 to 50 amino acids, possess a positively charged and amphipathic nature, and interact/insert directly with the components of LPA and LPS located on the bacterial membrane before lysis [78].

5.1. Antibacterial mechanism of AMPs

AMPs and their derivatives are one-of-a-kind compounds with a well-studied mechanism of action (MOA), and understanding their antibacterial mechanisms is necessary for the future development of therapeutic agents [75]. The antibacterial ability of AMPs is assisted by hydrophilic positively charged domains that interact with negatively charged microbial surfaces and the head groups of bilayer phospholipids, enabling penetration of the cell membrane [74]. The AMPs first gather at the bacterial cell surface, following initial electrostatic and hydrophobic interactions, and after reaching a sufficient concentration, they self-assemble and attack the bacterial membrane [75].

It is commonly assumed that the transmembrane pore (i.e., Barrel-Stave and toroidal model) and non-pore model (i.e., carpet model) are two possible MOAs of AMPs. As shown in Fig. 4 [79], the Barrel-Stave model assumes that AMPs are initially oriented parallel to the bacterial membrane but later inserted perpendicularly into the lipid bilayer for the formation of transmembrane pores [75]. In

Table 3. AMPs against MDR bacteria and their MIC value.

AMP	Sequence	Target MDR pathogens	MIC (μg mL^{-1})	ref
Dendrocin-ZM1	TTLRLNTLAYKVAWLVNVKAFWAAGRALKKVGR	*S. aureus*[a]	16	[86]
		VRSA[b]	32	
		E. coli[a]	16	
SAMA	GRLIDKIARKLVKKIQRFARKFF	*S. aureus*[a]	42.5	[109]
		E. coli[a]	14.2	
Dermcidin-1L	SSLLEKGLDGAKKAVGGLGKL	XDR[c] *A. baumanni*	16	[85]
	GKDAVEDLESVGKGAVHDVKDVLDSVL	PDR[d] *A. baumanni*	8	
Microcin J25 (MccJ25)	GGAGHVPEYFVGIGTPISFYG	Ciprofloxacin resistant *E. coli*	0.25	[110]
		enrofloxacin resistant *E. coli*	1.0	
		colistin resistant *E. coli*	0.5	
D-like (ΔM2)	[e]	*K. pneumoniae*[a]	8–16	[84]
		P. aeruginosa[a]	8–16	
CDPB11	VRNSQSCRRNKGICVPIRCPGSMRQ IGTCLGAQVKCCRRK	Colistin resistant *E. coli*	>200	[111]
			100	
		E. coli[a]	25	
		A. baumanni[a]	>200	
		P aeruginosa[a]	>200	
		K pneumoniae[a]		
WLBU2	RRWVRRVRRWVRRVVRVVRRWVRR	*A. baumanni*[a]	5.31–42.5	[112]
		K pneumoniae[a]		
Polybia MP-1	IDWKKLLDAAKQIL	*P aeruginosa*[a]	124	[113]
Linear-IRK	NH2-IRIKIRIK	MRSA[f]	16.6	[114]
BDT-4G	[e]	*P. aeruginosa*[a]	7.5	[115]

[a] Multidrug resistant bacteria.
[b] VRSA: vancomycin-resistant *Staphylococcus aureus*.
[c] XDR: extensively drug-resistant.
[d] PDR: pan drug-resistant.
[e] Not available.
[f] MRSA: Methicillin-resistant *Staphylococcus aureus*.

contrast, the toroidal model suggests that AMPs interact with the bacterial bilayer surface and bend the lipid structures constantly, resulting in lipid damage with the formation of a toroid of high curvature. Compared to the above-mentioned models, the carpet model postulates that AMPs prefer to cover the membrane surface in the form of a "carpet" before destroying bacterial membrane integrity [75]. The detergent model postulates that a disastrous change in the lipid membrane conformation is observed while adding a higher AMP concentration to bacterial strains.

5.2. Public resources of AMPs

Several databases have been designated for AMPs that can be used by researchers. They not only incorporate data on a large number of AMPs but also provide methods for forecasting AMP structures, activities, and toxicities [80]. Open-access databases that provide information on AMPs include The Data Repository of Antimicrobial Peptides (DRAMP 2.0) which contains general, patent, and clinical antimicrobial peptides (AMPs) with 19,899 entries [80]. The Antimicrobial Peptide Database (APD 2009) was expanded to APD3 and currently summarizes 2619 natural AMPs with known sequences and activities [81]. Moreover, the collection of antimicrobial peptides in CAMPR3 contains 10,247 sequences, 757 structures, 114 AMP family-specific signatures, and tools for AMP sequence and structure analysis [82]. Many computational and statistical algorithms have been developed to identify novel medicinal AMPs and have been discussed in a review article by Aronica et al. [83].

5.3. Examples of AMPs against drug-resistant bacterial pathogen

AMPs are promising candidates for the prevention and control of multidrug-resistant bacterial infections. The minimum inhibitory concentrations for bactericidal activity of AMPs against multidrug-resistant bacterial strains are summarized in Table 3. Sánchez *et. Al* [84] evaluated the inhibitory effect of a cecropin-like cationic peptide generated from cecropin D-like (ΔM2) against clinical isolates of

multidrug-resistant *K. pneumoniae* and *P. aeruginosa*, and its interaction with membrane models and bacterial genomic DNA. The broth microdilution test was used to determine *in vitro* antibacterial activity, indicating a MIC value of 8–16 μg/mL.

Farshadzadeh et al. investigated the potency of anti-biofilms *in vitro* and *in vivo* using dermcidin-1L (DCD-1L) against the highly drug-resistant PDR *A. baumannii.* DCD-1L showed MIC values of 16 μg/mL and 8 μg/mL against XDR- and PDR *A. baumannii*, respectively. Thus, DCD-1L may be a promising target for drug development in the antibacterial and anti-biofilm fields [85]. Seyedjavadi et al. [86] reported a novel antimicrobial peptide, dendrocin-ZM1, isolated from *Zataria multiflora Boiss*, which showed remarkable activity against multidrug-resistant *S. aureus*, *E. coli*, and vancomycin-resistant *S. aureus* with MIC values of 16, 16, and 32 μg/mL, respectively, with negligible cytotoxic and hemolytic activities.

6. Conclusions and future trends

In this review, we have highlighted recent studies on the detection and development of antibacterial reagents for antibiotic-resistant bacterial isolates. Compared to wild-type clinical bacterial pathogens, drug-resistant bacteria prefer to change their conformation or encode drug-resistant components through several antibiotic resistance mechanisms to defend against antibiotic invasion. Therefore, several analytical strategies (i.e., chemical/biochemical reactions, electrostatic attraction, and probe-free assays) have been developed for the sensitive and selective detection of specific drug-resistant bacterial strains, and are discussed herein. However, these methods have limitations, including being time-consuming, requiring sophisticated working procedures, moderate matrix effects, and narrow selectivity in clinical biopsies. Most importantly, the use of these protocols to detect various bacterial strains with the same drug-resistant behavior remains questionable. To solve this issue, molecular recognition elements (i.e., aptamers, antibodies, or microphages) for individual bacterial isolates or separation methods can be included in the sensing system.

To treat drug-resistant bacterial infections, biogenic AgNPs and AMPs may be two of the next waves of nano-antibiotics for the inhibition of drug-resistant bacterial growth. Despite the wide range of applications for silver nanoparticles, their antibacterial properties are the most utilized. Therefore, this has been effectively explored and used in numerous medical procedures. Studies have confirmed that biogenic AgNPs induce structural changes in the bacterial membrane, leading to the accumulation of NPs on the cell surface and the production of excessive ROS to kill the bacteria. However, the drawback of silver nanoparticles is their potential for toxicity due to AgNPs in higher concentrations being hazardous and can cause several health problems, and unpredictable changes in the blood cells, along with several ecological issues. Therefore, understanding both the molecular processes of toxicity and the interaction of AgNPs with the body is necessary for implementation in clinical applications [87]. In addition, bacterial resistance to AgNPs occurs during the accumulation of a high dosage of Ag nanomaterials. AMPs are derived from living organisms and provide broad-spectrum antibacterial properties with improved biosafety. Although cationic AMPs comprising positively charged amino acids (i.e., arginine and lysine) exhibit better antibacterial efficacy, they are less stable in serum proteases. Information from the AMP databases discussed above can be used to rationally design and replace amino acids with non-proteinogenic or D-form residues to increase the serum stability of AMPs with acceptable cytotoxicity to human cells. Collectively, the discovery of novel detection methods and next-generation antibacterial reagents may help reduce the growth and propagation of antibiotic-resistant pathogens worldwide.

Conflict of interest

The authors declare that there are no conflicts of interest.

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