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Rapid methods for antimicrobial resistance diagnosis in contaminated soils for effective remediation strategy

Cailing Zhou [a, b], Yuwei Pan [b], Shifu Ge [a], Frederic Coulon [b, **], Zhugen Yang [b, *]

[a] Key Laboratory of Energy Thermal Conversion and Control of Ministry of Education, School of Energy and Environment, Southeast University, NO. 2 Sipailou, Nanjing 210096, PR China
[b] School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, United Kingdom

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ABSTRACT

Antimicrobial resistance (AMR) in the environment is a global concern for public health and recent studies have shown that various soil pollutants (e.g. heavy metals, petroleum hydrocarbons) can cause the emergence of antibiotic-resistant bacteria and antibiotic-resistance genes in the soil. This emergence of AMR in soil is therefore prompting the research community for the development of rapid and real-time monitoring tools to better understand the source, fate and transfer pathway of AMR in contaminated soils. In this respect, the recent development of rapid sensors-based methods has been critically reviewed. The analytical performance of each sensing technique along with their advantages and limitations is further discussed to inform future development needs for the next generation sensors that would allow rapid and multiplexed detection of AMR in contaminated soils. By doing so, it would assist the decision making during remediation project and provide crucial insights into the risk assessment for contaminated sites.
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1. Introduction

Antimicrobial resistance (AMR) is a global concern which has attracted increasing attention since 2001, when the WHO issued their report on the need to take action to mitigate the emergence and the transmission of antimicrobial resistant microorganisms [1]. Antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) which are promoted by the inappropriate use of antibiotics, have been frequently detected in various aquatic and soil environments. This AMR emergence in the environment can seriously threaten the health of humans and animals [2,3]. In the U.S, it was estimated that 2.8 million people are infected with antibiotic-resistant bacteria or fungi each year and more than 35,000 people die every year due to ARB infection [4]. O'Neill (2016) further reported that there would be 10 million lives at risk per year and an enormous cumulative 100 trillion USD cost if no action is taken [5].

To date, a lot of attention has been devoted to wastewater treatment plants (WWTPs) as they have been shown to be hot-spots for the accumulation and dissemination of AMR in the environment [6,7]. Studies have also shown that WWTP effluents can still contain a high level of ARGs, even after disinfection [6]. In contrast, less attention has been devoted to sludge or biosolids while there are also potential sources of AMR especially for soil when either disposed to a landfill or reused as a soil conditioner and fertiliser for agriculture or remediation purposes [8–11]. Even though the soil is a natural habitat for the actinomycete and *Streptomyces*, whose members are the well-known antibiotic-producing species and present multidrug resistance [12,13], the anthropogenic agricultural and industrial activity-induced AMR exceeds the normal ecological soil balance. Since ARGs in the soil can indirectly threat human health through the food chain and dietary habits [14], this has now raised worldwide attention on the spread of AMR in soil [15–20]. In response, several studies on the prevalence of AMR in soil have been recently conducted reporting specific issues on contaminated soils, the influence of pollutant transfer and bioremediation strategies on AMR dispersion from contaminated sites [21–24]. However, not all studies corroborate similar findings and the main inconsistencies are that

* Corresponding author.
** Corresponding author.
E-mail addresses: f.coulon@cranfiled.ac.uk (F. Coulon), zhugen.yang@cranfield.ac.uk (Z. Yang).

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the application of manure or sludge can contribute to the AMR in soil [25,26] while some studies manifested no apparent correlation between them [27,28]; the bioaugmentation provides both the introduction of AMR in soil and the benefits of better soil remediation effect [29–31].

Further to this, most of the studies conducted to date are using traditional microbial and molecular methods such as microbial culture, microscopy, colony counting, etc. for AMR or ARB detection which are usually laboratory-based, time-consuming and require trained personnel. Even if using the later-developed methods such as polymerase chain reaction (PCR), reverse transcription-polymerase chain reaction (RT-PCR), loop-mediated isothermal amplification (LAMP), flow cytometry etc. to detect microorganisms and the related antimicrobial genes, we still rely on the laboratory-based techniques and require well-trained personnel,. To overcome these limitations, there is an urgent need for simple, rapid, accurate, and sensitive methods for the detection of AMR [32].

In the last decade, several rapid sensor-based methods have been developed including optical [33], electrochemical [34], mass sensitive, and microfluidic-based sensors [35]. They all have shown high sensitivity, specificity, easy-operation, and portability for the detection of inorganics (metal ions, sulfide, etc.), organics (antibiotics, pesticide, hydrocarbon, drugs, etc.), biomolecules (DNA, RNA, antibody, plasmid, etc.), microorganisms (bacteria, fungi), and viruses. However, to the best of our knowledge, there is to date only a very limited number of studies considering the environmental issues posed by contaminated soils and sustainable bioremediation practices as triggering the emergence of the potential new reservoir of antimicrobial resistance and how this can be tackled and monitored. Therefore, this review discussed the causes (i.e. pollutants), in addition to the well-known antibiotics, that can trigger the AMR in soil, and how the AMR emergence would be affected and transmitted into the soil through bioremediation methods aside from the water-sourced pathway, followed by the introduction of recent progress on rapid methods for AMR-related targets detection. The common characteristics of different rapid assays were compared and analysed to give a future strategy for developing rapid detection method allowing for simultaneous detection of AMR and associated contaminant in the soil to inform risk assessment and site remediation end point.

2. Pollutants triggering AMR in soil

The excessive use of antibiotics is the main reason for AMR into the soil via sludge or biosolids spreading to land. Knapp et al. [36] reported that concentrations of some specific ARGs in Dutch soils where biosolids application was used were 15 times higher in 2008 compared to values of 1970. Other studies also reported that AMR in the soil can be triggered by various conditions, such as the intrinsic soil physicochemical properties (e.g. pH, EC and redox), the carbon source content, the nutrient levels [37] and the chemical contaminants [38]. Thus, the exposure to pollutants from anthropogenic activities and how is this triggering AMR in the soil is becoming one of the most important concern to human health [24]. Recent evidence showed that besides the antibiotics, the pollutants such as heavy metals and PAHs in the soil can also exert positive selective pressure on multiple-antibiotic-resistant bacteria due to their attempt to resist to the stressors, thus prompting them to acquire ARGs and aggravating AMR phenomenon [39].

Therefore, in this review we highlighted how common contaminants found in contaminated sites, mainly heavy metals and petroleum hydrocarbons, exert potential pressure on soil microorganisms and AMR selection.

2.1. Heavy metals

In addition to antibiotics, heavy metals can stimulate AMR in the environment. Studies have shown that ARGs in soil could be innately affected by both geochemical conditions [40], and heavy metal contamination [38,41]. For example, the co-existence of antibiotics and Zn at subtoxic levels contributed to an increase of AMR of the culturable bacteria [42]. Similarly, high concentrations of Cu, Cd, and Ni in the soil can contribute to a high level of antibiotic resistance for ampicillin and sulphanilamide [43] and tetracycline [44]. Other studies showed that soil amendment with antibiotic-free manure can trigger ARGs selection in soil microbial communities due to the presence of high levels of heavy metals [45]. Knapp et al. [46] further found that residential soils, which were assumed to have no history of exposure to antibiotics, had higher relative ARG abundances when biosolid derived fertiliser with low metal concentrations was used for gardening activities. These findings clearly highlighted that even low metal concentrations can trigger both metal tolerance and antibiotic resistance in soil microbial communities and therefore suggesting a relationship between AMR and heavy metals. Studies have shown that metal resistance genes are generally found with ARGs on the same mobile genetic elements (like plasmids) [47] that are easily disseminated through horizontal gene transfer (HGT) [48]. This means the resistance genes can be easily transferred to other cells and even from dead cells.

Other studies also reported that the co-regulation by heavy metals and antibiotics might originate from the multi-drug resistant genes of the soil microorganisms (mainly efflux pump genes), which is also called cross-resistance mechanism and therefore facilitate AMR in the environment [49,50].

2.2. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) can also contribute to elevated AMR levels such as changing the abundance of both the intrinsic ARB and hydrocarbon degrading bacteria developing resistance to antibiotics [31]. Further to this, Chen et al. [51] showed that ARG abundance in PAHs-contaminated soils was approximately 15 times higher in soil heavily contaminated with PAHs compared to soil with lower concentration.

With regards to the environmental pressures exerted by PAH contaminated soils on AMR emergence, these remains largely unexplored compared to heavy metals. Recent research suggests that efflux pump is the dominant resistance mechanism involved for PAH contaminated soils where about 70% of studies reported resistance related to aromatic antibiotics such as acriflavine and fluoroquinolone [51]. Also, the Class I Integrons were attributed to ARG increase in the coastal microbiome due to conjugative transfer mechanism triggered by the presence of PAHs [52]. After ARG or Class I Integrons transmission, acquisition and expression, the soil indigenous bacteria could adapt to the new environment (i.e. contaminated environment) and exhibit AMR traits [53,54]. Also due to their mutagenic properties, PAHs can directly change DNA characteristics and/or trigger stress/repair systems that confer multiple resistant genes to the adapted microbes and thus contributing to AMR [55]. It is also worth pointing out that contaminated sites are often impacted by a wide range of chemical mixture, and therefore the co-presence of PAH and heavy metals is often found at contaminated site [31,56]. The presence of such complex chemical mixtures can further complicate the understanding of AMR emergence at site, as studies have shown an increase, similar level or even lower level due to cofounding factors and co-toxicity effects on soil microbial communities and lower opportunity for gene transfer between cells [57–59].

3. AMR transmission during bioremediation application

Recent move towards integrated and sustainable bioremediation practices for contaminated sites may contribute to the emergence, co-selection and transmission of antimicrobial resistance (AMR) in the environment [21] Amendment application to improve nutrient levels of contaminated soils and enhance microbial degradation of contaminants is a common practice but without some drawbacks, as it may impact upon the mobility and/or bioavailability of contaminants (i.e. heavy metals and PAHs) and subsequently on AMR emergence at site. Recent studies have looked into these issues as detailed in the following sub-sections.

3.1. Manure amendment

The use of animal manure for soil amendment is perceived as one sustainable approach for agricultural soil fertilisation and contaminated soil remediation where levels of nutrients are often depleted [60]. Besides, the slow-release nitrogen and phosphorus, and the additional organic content from the manure can biostimulate microbial activities and the degradation of petroleum hydrocarbons. For example, Liu et al. [61] reported higher degradation rate of petroleum hydrocarbons when pig manure was added to oily sludge compared to the untreated one. Poultry manures have also been used successfully for the bioremediation of diesel, lubricating oil or gasoline contaminated soils [61–65].

However, animal manures often contain antibiotics, ARB, ARGs, and in certain cases the levels are not insignificant. For example, tetracycline and sulfonamide resistance genes have been systematically detected in manure that were applied to agricultural soils in China [28,66–68]. Similarly, studies showed that long-term application of manure containing antibiotics triggered the amplification of ARGs thus increasing their diversity and abundance in agricultural soils. However, the prevalence of environmental AMR on contaminated soils amended with animal manure is still today largely ignored.

It is also worth pointing that not all the manure application would exert side-effect on the dispersal of ARGs and ARB in soil. Ghosh and LaPara (2007) found no increased chlortetracycline-resistant bacteria in soil amended with different tylosin- or chlortetracycline-containing manures [27]. Tang et al. [69] reported that even though the ARGs abundances in paddy soils increased over a decade of manure application, the increases were not significantly in other sites.

Another point is that previous researches mainly focused on a limited number of ARGs and therefore providing only a limited snapshot of the true environmental situation [27,45,66,69–71]. Some other studies also showed that unamended soils can also show higher ARGs prevalence compared to soils amended with treated pig manure [60]. Other studies have also highlighted that the presence of highly diverse microbial community may contribute to limit ARGs transmission in amended soils [71–74]. Hence AMR prevalence and dissemination in manure amended soils remains inconclusive [11].

3.2. Sewage sludge amendment

Similar to the application of manure, sewage sludge is also treated as a cheap and disposable fertiliser which could provide nutrients and microorganisms for bioremediation of PAH contaminated soil [75–77]. For instance, sewage sludge amendment to a 10,000 mg kg^{-1} diesel spiked soil resulted in 99% total petroleum hydrocarbons (TPH) degradation within 30 days [78]. In another study, it was shown that sewage sludge amendment to urban soil in Poland resulted in enhanced degradation of PAHs after a one-year application, [79]. Similar findings have been reported for hydrocarbon contaminated soils in Australia and Canada [80,81].

However, most studies published to date have largely ignored the potential prevalence of AMR on these sites.

3.3. Bioaugmentation

In a few instances, the rate of petroleum hydrocarbons biodegradation in the soil can be limited due to the number of hydrocarbon-degrading bacteria present in the soil. In such case, bioaddition or bioaugmentation of hydrocarbon degraders is a common strategy used to speed up the rate of degradation. However such practice in light of recent studies can contribute to the prevalence of AMR in contaminated soils [24,31,82]. For example, Máthé et al. obtained a collection of hydrocarbonoclastic bacteria that showed remarkable PAH degradation potential and tolerance to heavy metals [31]. They also found a significant correlation between antibiotic tolerance/hydrocarbon degradation ability and heavy metal/antibiotic tolerance ability for strains isolated from sites co-contaminated with heavy metals and hydrocarbons, while these correlations were not detected in soils solely contaminated with hydrocarbons. Similarly, Benedek et al. developed a bacterial consortium containing *Rhodococcus qingshengii* BBG1 and *Pseudomonas fluorescens* BBN1 to degrade hydrocarbons in presence of Cu^{2+}, Pb^{2+}, Zn^{2+} and under a wide range of pH [83]. Again, they observed that the strains developed antibiotic resistance over time. While it is difficult to say at this stage, whether developing antibiotic resistance ability can pose a long-term issue or not at contaminated sites, some researchers held the view this was a positive effect for bioremediation as the hydrocarbon-degrading bacteria can withstand degradation activities in a highly polluted environment [29,30]. Others instead recognised that soil bioremediation strategies should consider more than just the removal or decrease of pollutants and therefore the prevalence of AMR should be systematically considered and investigate.

In addition, microbial bioremediation is suggested to be tested for antimicrobial susceptibility and confirmed with low or none antibiotic resistance before use [21]. For instance, *Pseudomonas aeruginosa* (*P. aeruginosa*) which are tolerant to multiple antibiotics and heavy metals because of their efflux pump proteins, are not recommended to be used in bioremediation. Kaszab et al. detected multi-resistant strains of *P. aeruginosa* isolated from eight PAH contaminated soil samples of the total 26 sites in Hungary [82]. Such bacteria are more useful to the sorption of antibiotics or heavy metals rather than the soil remediation. On the contrary, those bacteria without antibiotic resistances such as *Acidovorax temperans* [84] would be a very good choice for bioremediation.

3.4. Disposed biosolids

Generally, the pre-treated biosolids are characterised with better features like less antibiotic residues, pathogenic organics and ARGs [85]. Composted manure and sludge and pyrolysed biochar are the main typical disposed biosolids for soil remediation.

3.4.1. Composted products

The composted products from manure usually contain fewer antibiotic residues during the process of transferring degradable organics to stable humus [86]. They generally hold more abundant nutrients and can increase soil water retention, heat preservation, air permeability and fertiliser retention. Besides, the high temperature (usually about 60–70°C) generated during the composting process could disinfect some pathogens, worms and weed seeds in the original materials thus reducing ARB and ARGs in the manure. Therefore, composted sludges or manures have been the popular

bioremediation strategy for hydrocarbon-contaminated or the agricultural soils.

Ros et al. used fresh and composted sludge for the bioremediation of hydrocarbon-contaminated semi-arid soil [87]. After the cultivation for 8 months, they found that fresh sludges held the highest hydrocarbon-degrading rate of 46% while the rate for composted sludge was only 36% compared with the controlled one of 31%. Besides, the highest bacterial and fungal population were also observed in the fresh sludge treated soils. While after a 126-day greenhouse experiment of PAH removal in soil by sewage sludge or composted sludge, better degrading efficiencies of PAHs were found in compost soil [75]. However, situations were different with various soil/amendments ratio in the research of Namkoong et al. who wished to find the appropriate organic amendments mixing ratio for the increase of diesel oil degradation [78].

3.4.2. Biochar

Biochar is a carbon-rich solid derived from biomass (such as wood, leaves, dead plants, animal bones, dung, etc.) through the pyrolysis and has been widely applied in soil remediation [88]. Unlike manure, sludge, compost and organic degrading bacteria, the thermal pyrolysis under high temperature of biochar contributes to fewer risk for it to introduce microorganisms, ARB or ARGs into the soil when it is used as soil amendments. Due to its abundant carbon fibre and the functional group which could provide the nutrients and make an effect with organic and heavy metals, biochar has also been utilised for pollutants reduction in the environment such as ARGs and antibiotics [89–94]. Moreover, the carbon catabolic capacity of the biochar may also enhance the soil fauna abundance [95] which could alleviate the AMR dissemination in soil. Also, biochar is now usually added into other remediation material for better modifying the characteristics of amendments. The mobility and bioavailability of fluoroquinolones, fenicols and sulfonamides can be reduced by the biochar mixtures with manure [89]. To alleviate the side-effect of sludge as a biosolid compost that is imposing outer microorganism into the soil which could contribute to AMR, Liu et al. used 5% added biochar into the sludge composting for nitrogen transformation and immobilizing heavy metals [96].

Even though biochar has many superiorities than manure, sludge and bacteria in the soil remediation, the heavy metal content in it should be paid attention as biochar is the stimulus for AMR in soil [97]. Pyrolysis can reduce the most mass of biochar and concentrate the heavy metal concentration. Whereas, as various faeces-derived biochar has been widely used recently in farmland due to the characteristics of waste reuse and the high sorption performance for heavy metals, it's a potential risk to trigger the evolution of ARGs in the environment. Altogether, no matter what the effects of bioremediation methods on the AMR in contaminated soil, we should pay close attention to the disseminating pathways and affecting factors.

4. Rapid detection methods development for AMR and associated contaminants

Traditional detecting methods for microorganisms are microbial culture-based methods. These methods are typically time-consuming and can take hours to days. They also often require well-trained users. Another limitation is the identification of pathogens that are only culturable. The alternative way is the molecular method, such as polymerase chain reaction (PCR) and isothermal amplification [98–100]. PCR-based technologies such as real-time PCR, reverse transcriptase PCR, multiplex PCR, droplet digital PCR can all achieve multiple functions and goals for the detection of pathogens. However, they still need professional technicians and remain several tricky issues such as the precision reduction with low DNA concentration, the incorrect products sometimes when contaminated, the complicated pre-treatment procedures from original samples, etc.

Recently, rapid sensors including optical, electrochemical as well as microfluidics have been developed to detect pathogens, antibiotic resistance genes, and contaminants. Generally, most rapid methods focused on the detection of antibiotics, heavy metals, and the foodborne or iatrogenic pathogens, while it's still challenging for the detection of hydrocarbons. The rapid methods have a clear potential for multiple targets detection (such as ARB, ARGs, antibiotics, heavy metals, etc.), and can be eventually integrated into a single device.

4.1. Optical sensors

Optical sensors are widely applied for various analytes detection through the transducers of colourimetric, fluorescence, Raman spectroscopy (RS), surface plasmon resonance (SPR), etc. Those optical sensors combined with receptors (e.g. antibody, aptamers) as the biorecognition element usually have advantages of simplicity, sensitivity, selectivity and specificity. Hence, we discussed several main optical sensors.

4.1.1. Colourimetric sensors

Colourimetric sensors have attracted increasing attention due to their simple procedure, rapid response, low-cost and visual readout. The target signal is recognised through colour changes which can be detected by naked eye thus making the colourimetric sensors portable for on-site detection. Furthermore, colourimetric sensors have been combined with various readout devices to enhance their reading efficiency and accuracy [101]. The key feature of colourimetric sensors is the chromogenic substrate and catalytic enzyme. For example, the horseradish peroxidase (HRP) and G-quadruplex are the two commonly used catalytic enzymes due to their high catalytic efficiency and substrate specificity [102]. Those colourimetric sensors combined with peroxidase activity can provide an amplificated colour-change signal for the detection of various analytes, including antibiotics, heavy metals, pathogens, etc. While several types of nanoparticles (NPs) like gold nanoparticles (AuNPs) have been used for the modification of colourimetric sensors recently. Their excellent optical and electrical advantages, including large surface area, great quantum yield, high absorption coefficient, great luminescence and conductivity [103] make them the typical material for colourimetric sensors. The colour of AuNPs can be changed from red to blue or purple once they aggregate, which is treated as the signal of analytes recognition with ready readout by naked eyes. Generally, aptamers can be easily bonded to the surface of AuNPs to protect them from aggregation induced by ions or other materials. While they will be separated from the AuNP under the occurrence of analytes and bind to the target, contributing to the aggregation of AuNPs and colour changing [102]. Such colourimetric sensors can be used for a range of analytes based on this principle such as metals, antibiotics and microorganisms. For example, Gan et al. [104] used an aptamer functionalised AuNPs for the in-situ detection of cadmium in high salt solutions (Fig. 1A). This colourimetric sensor can detect Cd^{2+} in the range of 2–20 $\mu g\ L^{-1}$ with a detection limit (LOD) of 1.12 $\mu g\ L^{-1}$. Due to the shielding of electrostatic repulsion force exerted by the salt, AuNPs aggregate with Cd^{2+}, and the presence of the aptamer avoids its aggregation. However, the specific interaction between Cd^{2+} and the aptamer will attenuate the stability of AuNPs after the adding of Cd^{2+} and causes the solution colour change. Similar sensors have been developed for other metal saltsand metalloids such as Fe^{3+}, Hg^{2+}, As^{3+}, with high sensitivity and selectivity

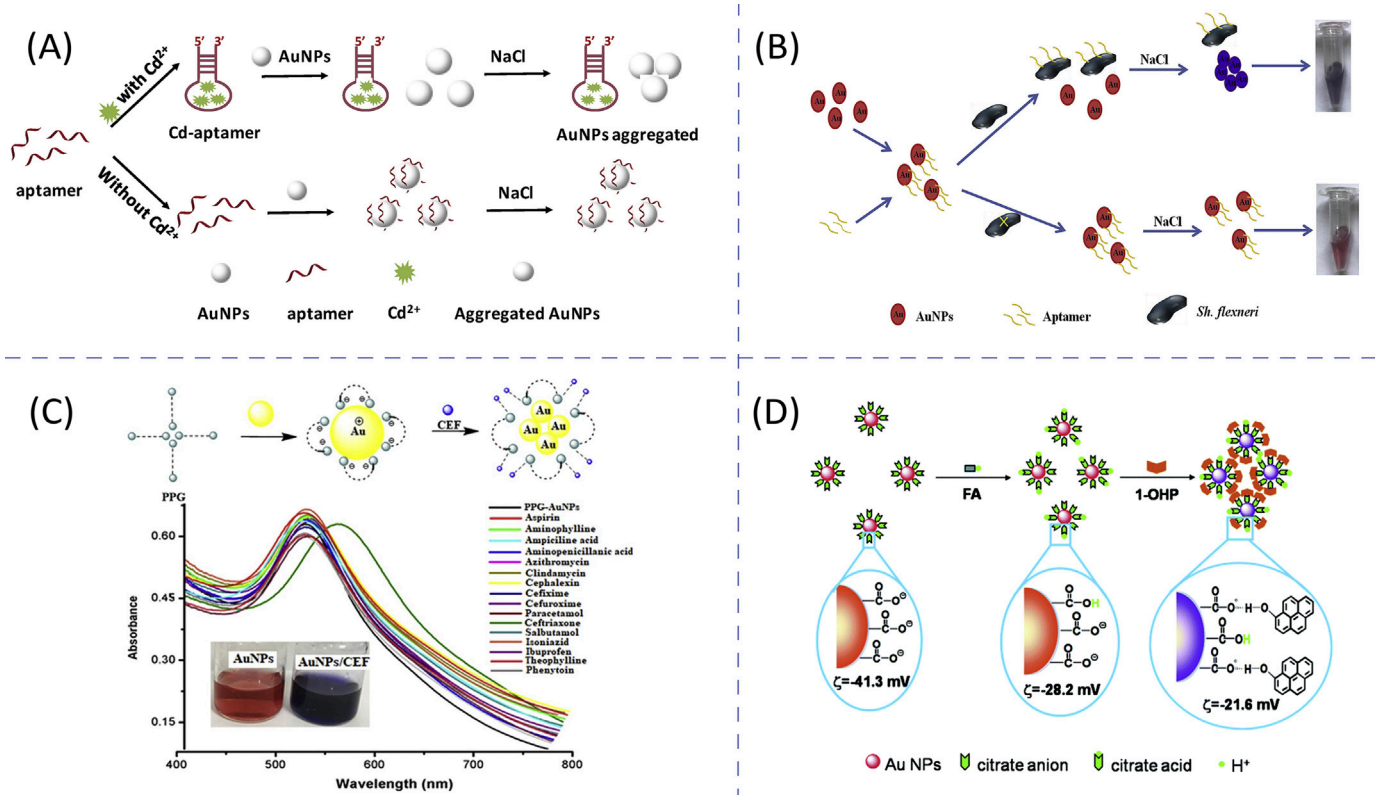


Fig. 1. (A) Detection principle for Cd^{2+} based on AuNPs using colourimetric aptasensor [104]; (B) Colourimetric aptasensor detection scheme for *Sh. flexneri* detection [33]; (C) UV–vis spectra after adding 0.1 mM antibiotic solution into PPG-AuNPs, and the colour refers to the colour change after adding ceftriaxone [108]; (D) Schematic diagram of colourimetric sensor for 1-OHP [109].

[105–107]. Colourimetric sensors can also be used for the detection of microorganism. For instance, Feng et al. [33] developed a colourimetric aptasensor combined with AuNPs for the detection of *Shigella flexneri* (*Sh. flexneri.*) (Fig. 1B). The target bacteria induce the release of aptamer immobilised on the AuNPs due to its high binding affinity between the specific aptamer and *Sh. flexneri.*, which leads to the aggregation of AuNPs after adding NaCl and changes the solution colour. The concentrations of *Sh. flexneri* can be detected within the range from 10^2 to 10^6 CFU mL^{-1} with the LOD of 80 CFU mL^{-1} under the optimised conditions in 20 min or less. Raja et al. [108] developed a colourimetric assay using Poly(-propylene glycol) (PPG) stabilised AuNPs for the detection of ceftriaxone (Fig. 1C). In their work, the colour of AuNPs changed from red to blue in the presence of ceftriaxone and the concentration ranged from 0.1 to 100 mM. In another study, Hu et al. [109] utilised AuNPs assay for the direct quantification of 1-hydroxypyrene (1-OHP) in urine samples with a LOD of 3.3 nM (Fig. 1D). The AuNPs were initially coupled with citrate and then formic acid was added to stabilise the AuNPs. Once 1-OHP was added, it interacted with the carboxylic anions on AuNPs due to the hydrogen bonds, leading to the non-crosslinking aggregation of AuNPs within 5 min and changing the colour from red to violet blue. Apart from the AuNPs, other nanomaterials including carbon nanotubes (CNTs) and quantum dots (QDs) [110,111] have been widely explored for colourimetric sensors development.

Even though this colourimetric assay was aimed for a single pathogen, it provides a generic platform that can be modified by replacing the aptamers to be implemented for the detection of a range of ARB in the environment and other targets. Therefore, identifying specific aptamer which has a high affinity to either the targeted chemicals or the targeted microbial strains is the key for future sensor development. For more convenient reading out, smartphones have been widely utilised for accurate on-site detection of antibiotics [101], heavy metals [104,107,112], pathogens [113], etc. which significantly improve the convenience and accuracy of colourimetric detection.

4.1.2. Fluorescent sensors

Fluorescent sensors are powerful analytical technologies because of their simple operation, rapid analysis, good reproducibility and high sensitivity. The signal sources used for fluorescent sensors are either organic dyes or nanomaterials such as QDs, AuNPs, graphene oxide (GO), providing notable advantages such as broader absorption, narrower and symmetric emission band, stable fluorescence, and higher resistance to photobleaching.

Nanomaterials can provide fluorescent signals that can be used for the recognition of conformation changes of bioreceptor (e.g. DNA aptamer) when the analytes were targeted. Yu et al. [114] presented a one-step strategy where they functionalized gold nanoclusters (AuNCs) with the antibiotic vancomycin (Van) and a nucleic acid aptamer for the selective detection of *Staphylococcus aureus* (*S. aureus*) within 30 min (Fig. 2A). The dynamic range was between 20 to 10^8 CFU mL^{-1} and the LOD was 10 CFU mL^{-1}.

In addition to the utilisation of AuNPs alone, the combination of AuNCs and magnetic beads (MB) is also commonly used for the detection of pathogens. The AuNCs are generally stabilised with some antibiotics for the fluorescent detection of targets. The magnetic beads are usually coated with aptamer for the capture and enrichment of targets. For example, Cheng et al. [115] utilised the Van-stabilised AuNCs (AuNCs@Van) and aptamer-coated MB (Apt-MB) to detect *Staphylococcus aureus* (*SA*) in single culture sample and complex culture samples (Fig. 2B). The Apt-MB was first

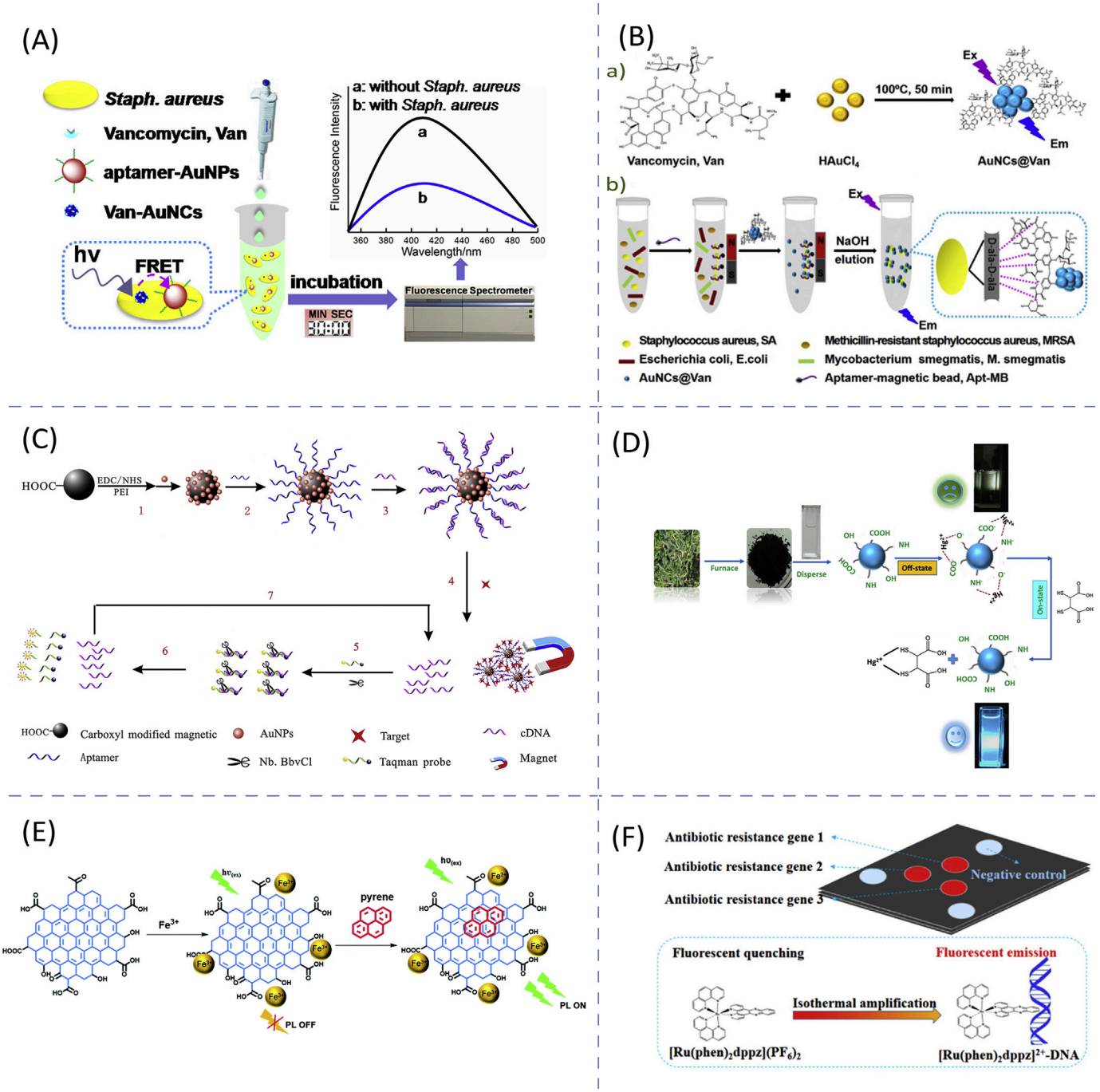


Fig. 2. (A) Schematic illustration of vancomycin based aptasensor for *Staph. Aureus* [114]; (B) Schematic Illustrations of a) Preparation of AuNCs@Van, and b) Detection process for *SA* in using Apt-MB and AuNCs@Van [115]; (C) Schematic illustration for the detection of ampicillin amplified strategy for fluorescence signal using AuNPs/MBs with a nicking enzyme [116]; (D) Detection principle of turn-off/on fluorescence probe based on the green CQDs [117]; (E) Schematic illustration of the working mechanism of GQDs with Fe^{3+} for fluorescence quenching and the turn-on mode with pyrene [118]; (F) Schematic illustration of the interaction of different ARGs [119].

added into the samples containing *SA* to capture them by magnetic enrichment, and then the AuNCs@Van were added into the sample and bound on the Apt-MBs. After the elution with NaOH for eliminating Apt-MBs on the surface of *SA*, the left SA@AuNCs@Van was detected and recorded by the fluorescence signal. In this work, the AuNCs@Van were prepared in a simple one-step method which simplified previously published procedures. This new approach also allowed to quantify *SA* in the range of 32–10^8 CFU mL^{-1} with a LOD of 16 CFU mL^{-1}, enabling to detect SA as low as 70 CFU mL^{-1} in multi-bacteria samples. Similar strategies can be applied to the detection of other analytes. Luo et al. [116] developed a fluorescent aptasensor utilizing AuNPs modified magnetic bead composites (AuNPs/MBs) and nicking enzyme for antibiotic detection (Fig. 2C). AuNPs/MBs were synthesised with polyethyleneimine (PEI) and specific aptamer which bonded with complementary DNA (cDNA) for the detection of ampicillin. After adding ampicillin, partial cDNA was free from the aptamer due to the affinity between aptamer and ampicillin. Next, the cDNA initiated the cycle of fluorescence signal amplification using a nicking enzyme with TagMan probes. In this assay, the concentration of ampicillin can be sensitively detected in

the range of 0.1–100 ng mL^{-1} with a LOD of 0.07 ng mL^{-1}. The sensor combined with AuNPs and MBs presented a better performance than only using MBs as they can magnify the separation capacity with strong covalent bio-conjugation.

QDs are also widely-used materials for the detection of pathogens [120,121], heavy metals, antibiotics [117,122–126] and hydrocarbons [118]. For instance, Pourreza and Ghomi developed fluorescent aptasensor based carbon quantum dots (CQDs) exhibiting a high quantum yield of 5% blue luminescence with excellent and facile performance for the detection of Hg^{2+} and chemet (Fig. 2D) [117]. In the presence of Hg^{2+}, the CQDs were bonded with Hg^{2+} and the fluorescent response was off. In contrast, in presence of chemet, the fluorescence was detectable within 2 min with high affinity to Hg^{2+}. The concentration of Hg^{2+} and chemet can be detected in a linear range from 5 to 500 ng mL^{-1} and 2.5–22.5 ng mL^{-1} with a LOD of 1.26 ng mL^{-1} and 1.4 ng mL^{-1}, respectively. Since the CQDs was derived from *Prosopis juliflora* leaves, this assay offers a low-cost and sustainable sensor on the market place.

Graphene quantum dots (GQDs) provide additional advantages such as higher biocompatibility, photostability, and larger surface area, which allow wider sensing applications such as the detection of petroleum hydrocarbons. For example, Nsibande and Forbes developed a fluorescent assay with GQDs and associated ferric ions for the detection of pyrene in lake water samples which was also based on the "turn-off-on" strategy (Fig. 2E) [118]. The addition of ferric ions acted as fluorescent quencher that turned off the fluorescence emission at first while turned on in the presence of pyrene through π-π stacking. Graphene oxide (GO) and citric acid (CA) were used as the two sources of GQDs in this study showing a linear range from 2 to 10^{-6} mol L^{-1} with a LOD of 0.325 × 10^{-6} mol L^{-1} and 0.242 × 10^{-6} mol L^{-1}, respectively. This assay was also prepared from environmentally friendly material with simple synthesis approach and showed high reproducibility.

Similar turn-on/off strategy can be used for the detection of many other analytes such as ARGs. Li et al. [119] developed a paper-based chip utilising loop-mediated isothermal amplification (LAMP) technology to detect genes in 1 h (Fig. 2F). $[Ru(phen)_2dppz]^{2+}$ was chosen as the light switch molecule for the turn-on fluorescent detection of *mecA* with a LOD of 100 copies. This assay simplified the detecting process by embedding the amplification reagents in advance and intercalating the $[Ru(phen)_2dppz]^{2+}$ to remove the washing procedure.

4.1.3. Raman spectrometry-based sensors

Surface-enhanced Raman scattering (SERS) based on light scattering has the potential for rapid detection of pathogens, antibiotics, metals, and hydrocarbons in soil. Main advantages of SERS sensors are high sensitivity, low photo-bleaching, rapid response time, simplicity and multiplexing. Owing to their excellent characteristics, SERS sensor is one of the most promising rapid methods for various analytes detection such as ultra-trace elements, organics, inorganics, microbial cells and viruses [127,128].

Pang et al. [129] developed an Aptamer-Fe_3O_4@Au magnetic nanoparticles (AuMNPs) sensor for the detection of various bacteria (Fig. 3A). AuNPs were modified by mercaptobenzoic acid (MBA) and conjugated with Van for the intense Raman signal detection. The aptamer-Fe_3O_4@Au MNPs were first synthesised to form a core (Fe_3O_4)-shell (Au) structure and then modified with mercapto-undecanoic acid (MUA) to get surface carboxylation. As Fig. 3A illustrated, Aptamer-Fe_3O_4@Au was first added into *S. aureus* cultures and then incubated to capture the targets and enrich them. The Au-van tags were then added and incubated. Finally, the Fe_3O_4@Au/bacteria/SERS tag complexes were detected by Raman signals. This assay could detect *S. aureus* within a total 50 min in the concentration range from 10 to 10^7 cells ml^{-1} with a LOD of 3 cells ml^{-1}.

AuNPs based SERS biosensors can also be used for antibiotics detection [128,133–135]. For example, Li et al. [130] developed a SERS-based magnetic nanospheres-targeting aptasensor for tetracycline (TTC) detection. The magnetic nanospheres (MNs) were composed of magnetite colloid nanocrystal clusters (MCNCs) conjugated with an aptamer and combined with polymethacrylic acid (PMAA). The resultant Au/PATP/SiO_2 (APS) acted as the Raman reporter (Fig. 3B). By immobilizing the APS with MNs, the composite MNs-targeting aptasensor was prepared by mixing the aptamers and cDNA on the surface of APS. After the addition of TTC, it bound on the aptamer and released cDNA-APS which were then produced strong SERS signals for the recognition of TTC. The concentration of TTC could be detected in a linear range from 0.001 to 100 ng mL^{-1} with a LOD of 0.001 ng mL^{-1}. In this assay, the high saturation magnetization of MNs can facilitate the easy and rapid magnetic separation for the further detection of targets in the supernatant. Besides, the cDNA on the APS can amplify the Raman signals to provide a strong SERS intensity for better recognition of TTC. Besides, the highly sensitive and well selective SERS aptasensor can still work under generally coexisted interferences.

Similarly, the AuNP-SERS sensors combined with other substances can be used for heavy metal detection [127,131,136–138]. For instance, Xu et al. [131] developed a rapid SERS assay for Pb^{2+} detection based on Ag-coated AuNPs of core-shell nanostructure modified with L-cysteine. Upon the formation of nanoparticle aggregation, the L-cysteine-functionalised Au@Ag probes with Raman labelling molecules (Au@AgNPs) were used to detect Pb^{2+} after reacting for 30 min with a linear range from 5 pM to 10 nM and an unprecedented LOD of 1 pM (Fig. 3C). Except for this detection mechanism, there are many other strategies of SERS sensors for heavy metals detection such as the "turn-on", "turn-off" mechanism, etc. [139].

The rapid on-site detection of PAHs remains challenging. Unlike colourimetric and fluorescence sensors, SERS sensors have a significant advantage for hydrocarbon detection [140–145]. For example, Zhou et al. [132] developed a SERS assay for the detection of the 16 USEPA PAHs using the Ag nanoparticles (AgNPs). Briefly, the 16 PAHs were extracted from water samples using liquid-liquid extraction (LLE) for 15 min (Fig. 3D). After solvent volatilization of the organic phase, the PAHs were eluted out to be ready for the detection by Ag-SERS sensors. The LOD values of anthracene, pyrene, and benzo[a] pyrene were 100, 50, and 5 ng L^{-1}, respectively. According to the number of aromatic rings for each molecule, these PAHs can be detected by this assay at concentrations from 100 to 0.1 μg L^{-1}.

Additionally, there are other optical technologies, including Surface plasmon resonance (SPR), and chemiluminescence. SPR is a sensitive analytical method which can detect minor changes at the interface between two materials. SPR sensors have the potential for various analytes detection, including antibiotics [146–149], genes [150–152], metals [153–156] and pathogens [157–162]. For instance, Zhang et al. [163] utilised Fe_3O_4 nanoparticle cluster (NPC) modified aptamer for signal amplification and detection of *Listeria monocytogenes* (*L. monocytogenes*). After adding the Fe_3O_4 NP or Fe_3O_4 NPC into the sample, they can identify the concentration of *L. monocytogenes* through the intensity of colour by naked eyes. The colour intensity was higher in the samples used with Fe_3O_4 NPC than those used with Fe_3O_4 NP. This simple and rapid detection method can quantify *L. monocytogenes* within a range of 5.4 × 10^3–10^8 CFU mL^{-1} and the LOD is 5.4 × 10^3 CFU mL^{-1} which can be completed in 145 min. We summarized selected optical sensors for various analytes detection in Table S1.

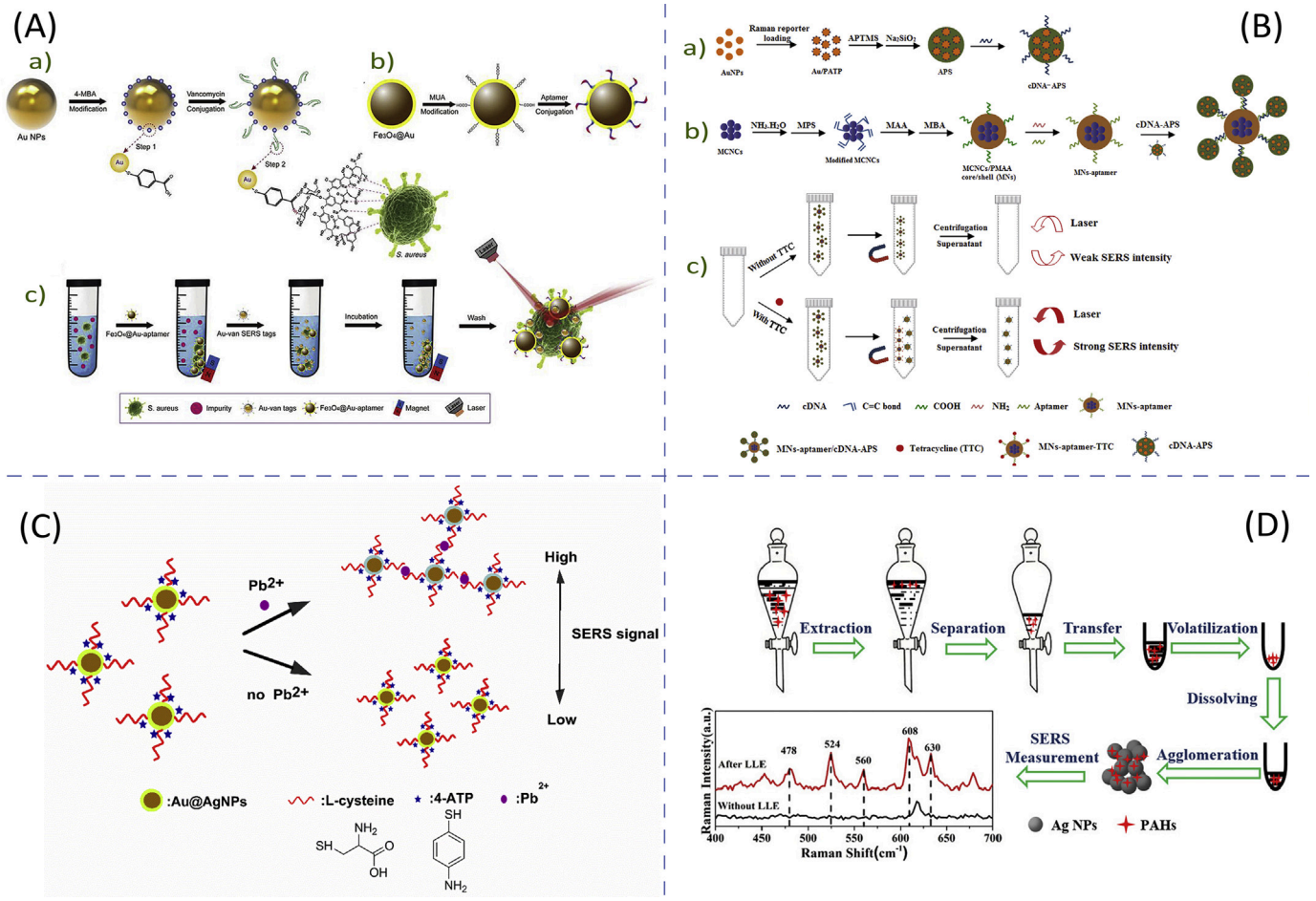


Fig. 3. (A) Schematic illustration of the synthesis of Au-Van SERS tags and aptamer-modified Fe_3O_4@Au MNPs, and the detecting procedure for *S. aureus* via the dual-recognition SERS biosensor [129]; (B) Schematic illustration of assay preparation and aptasensing for tetracycline [130]; (C) The detection principle and procedure of Pb^{2+} based on the aggregation strategy of Au@AgNPs [131]; (D) Schematic illustration of LLE-SERS procedure for detection of PAHs [132].

4.2. Electrochemical sensors

Electrochemical sensors have exclusive advantages of rapid response, low cost, and small size which are easy to be integrated with other electrical devices [103,164]. Furthermore, they only need a small dose of samples and do not require fluorescent materials, therefore avoiding the need for cleaning and reducing analysis cost. Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) have been widely reported for the rapid detection of antibiotics, metals/metalloids and pathogens. The development of surface chemistry on the electrode is the key for electrochemical sensors especially nanomaterials such as CNTs, graphene, immunomagnetic nanoparticles or beads which have been extensively used for coating inkjet-printed platforms and screen printed electrodes (SPEs).

Besharati et al. [165] used nano-porous glassy carbon electrode (NPGCE) as they are optimal electrodes for enzyme-based biosensor for the detection of tetracycline (TC) resistance. A TC degrading enzyme, TetX2 was immobilised on NPGCE using polycation polyethyleneimine (PEI) as the electrode (Fig. 4A). In the presence of TC, the oxygen reduction peak current was detected, showing a linear range from 0.5 to 5 μM with a LOD of 18 nM. Other developments of CV such as the fast-scan cyclic voltammetry (FSCV) are emerging for the detection of various analytes. The technique that FSCV combined with carbon-fibre microelectrodes (CFMs) has a rapid response to analytes concentration with minimal interference to the analytical medium. For instance, Yang et al. reported a highly selective measurement for subsecond Cu(II) using FSCV with covalently modified CFMs. In their approach, the binding of Cu(II) was enhanced by covalently grafted ionophore while the chemical blocking on the non-selective surface adsorption sites prevented the binding of other metals [166]. Therefore, Cu(II) could be detected in a complex medium with negligible interference.

Melo et al. developed a rapid and sensitive amperometric sensor for *Salmonella Typhimurium* [170]. The sensor consisted of a cysteamine monolayer modified gold electrode combined with protein A to immobilise the anti-Salmonella antibody, can detect as low as 10 CFU mL^{-1} *Salmonella* within 125 min for the final detection time.

The DPV measurement using electroactive indicators provides an effective and simple detection for various targets. GO is one of the most popular materials for electrode modification due to the π−π interactions between GO and aptamers. For instance, Shahrokhian and Ranjbar designed a hollow porous structure zeolitic imidazolate Framework (HZIFs) functioned by phenolic acids [167]. The aptamer and ferrocene-graphene oxide (Fc-GO) were immobilised on the HZIFs separately, and Fc-GO was coupled with DPV as the indicator for the analytes (Fig. 4B). In the presence of *Pseudomonas aeruginosa* (*P. aeruginosa*), the aptamer was bound to specific epitopes of *P. aeruginosa* thus releasing Fc-GO from the electrode surface. Through the signal-off strategy, this sensor showed a linear detection of *P. aeruginosa* from 1.2×10^1 to 1.2×10^7 CFU mL^{-1} with

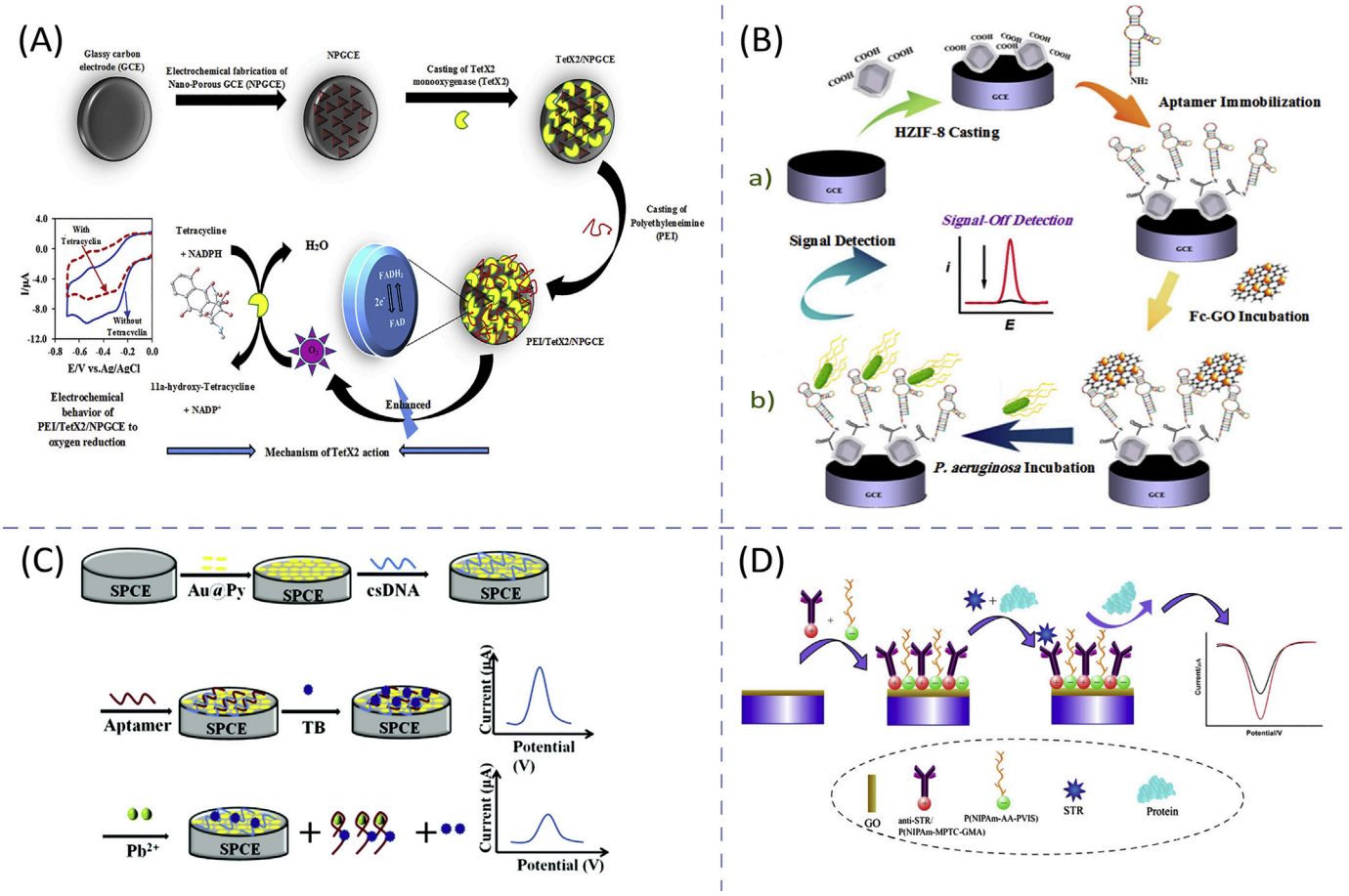


Fig. 4. (A) Preparation of enzyme-based nano-biosensor for the detection of tetracycline using CV [165]; (B) The preparation of aptasensor for the detection of *Pseudomonas aeruginosa* using DPV signal-off [167]; (C) The fabrication of aptasensor for the detection of Pb^{2+} using DPV [168]; (D) The fabrication procedure and detecting principle of the electrochemical biosensor for streptomycin using DPV [169].

a LOD of 1 CFU mL^{-1}. A similar strategy was also utilised for the detection of heavy metals, using AuNPs and polymers modified electrode [168] (Fig. 4C). In the presence of Pb^{2+}, the aptamer was attached to Pb^{2+} and released from the electrode, reducing the current signal. The detection range of Pb^{2+} was between 0.5 and 25 ppb with a LOD of 0.6 ppb. This detection method was even successfully applied to detect Pb^{2+} in soil.

Except for the signal-off or signal-down strategy [167,171], the signal-up strategy has been applied in the detection of pathogens [172]. Zou et al. developed a new sensor, where AuNPs were well-distributed on a polypyrrole-reduced graphene oxide (PPy-rGO) composite to assemble and fabricate a sensor for the detection of *E. coli* K12 [172]. This nanocomposite with a high surface area has good biocompatibility and conductivity. The linear range toward *E. coli* K12 was from 1.0×10^1 to 1.0×10^7 CFU mL^{-1} and the low LOD was 10 CFU mL^{-1}.

In most cases, the assays can commonly be polluted by the complex in the real samples. The development of those technologies of antifouling sensors has attracted the focus of researchers. He et al. developed a novel antifouling electrochemical sensor for antibiotics in complex media [169]. Two poly(N-isopropylacrylamide) microgels were utilised here for the detection of streptomycin (STR). The zwitterionic liquid 1-propyl-3-vinylimidazole sulfonate (PVIS) and glycidyl methacrylate (GMA) modified microgels were prepared for the specific recognition of anti-STR and antifouling, respectively (Fig. 4D). A decrease of current density was reflected by the electrochemical signals in the presence of antigen and anti-STR after the incubation of 35 min. The detecting linear range for STR was from 0.05 to 100 ng mL^{-1} with a LOD down to 1.7 pg mL^{-1}. In this assay, the Zwitterionic liquid-modified microgels with the ability to form stronger hydration presented an excellent anti-fouling ability which provided the possibility for this assay of low-fouling and sensitive detection for other analytes in complex media.

Electrochemical impedance spectroscopy (EIS) sensors used different aptamers to detect various antibiotics. For example, Wang et al. utilised the condensation polymerization between melamine and 1,3,6,8-tetrakis(4-formylphenyl)pyrene (TFPPy) forming an organic framework (COF) as a novel detecting assay (Py-M-COF) [173]. The main advantages include big pore cavities, large specific surface area (495.5 m^2 g^{-1}), extended π-conjugation framework, rich functional groups and nanosheet-like structure promoting a good scaffold to immobilise aptamers for antibiotics detection (Fig. 5A). After the binding of two aptamers for enrofloxacin (ENR) and ampicillin (AMP), the electrochemical results provide a sensitive reaction in the presence of each targeted antibiotic. This assay has an extremely low LOD of 6.07 and 0.04 fg mL^{-1} for the detection of ENR and AMP, respectively. By changing the relevant aptamers, other analytes can also be sensitively detected by this sensing platform. Similarly, such sensor can be modified with other DNA-based specific aptamers for the detection of heavy metals and pathogens. For example, Abu-Ali et al. reported an electrochemical sensor for rapid detection of Hg^{2+} and Pb^{2+} using the screen-

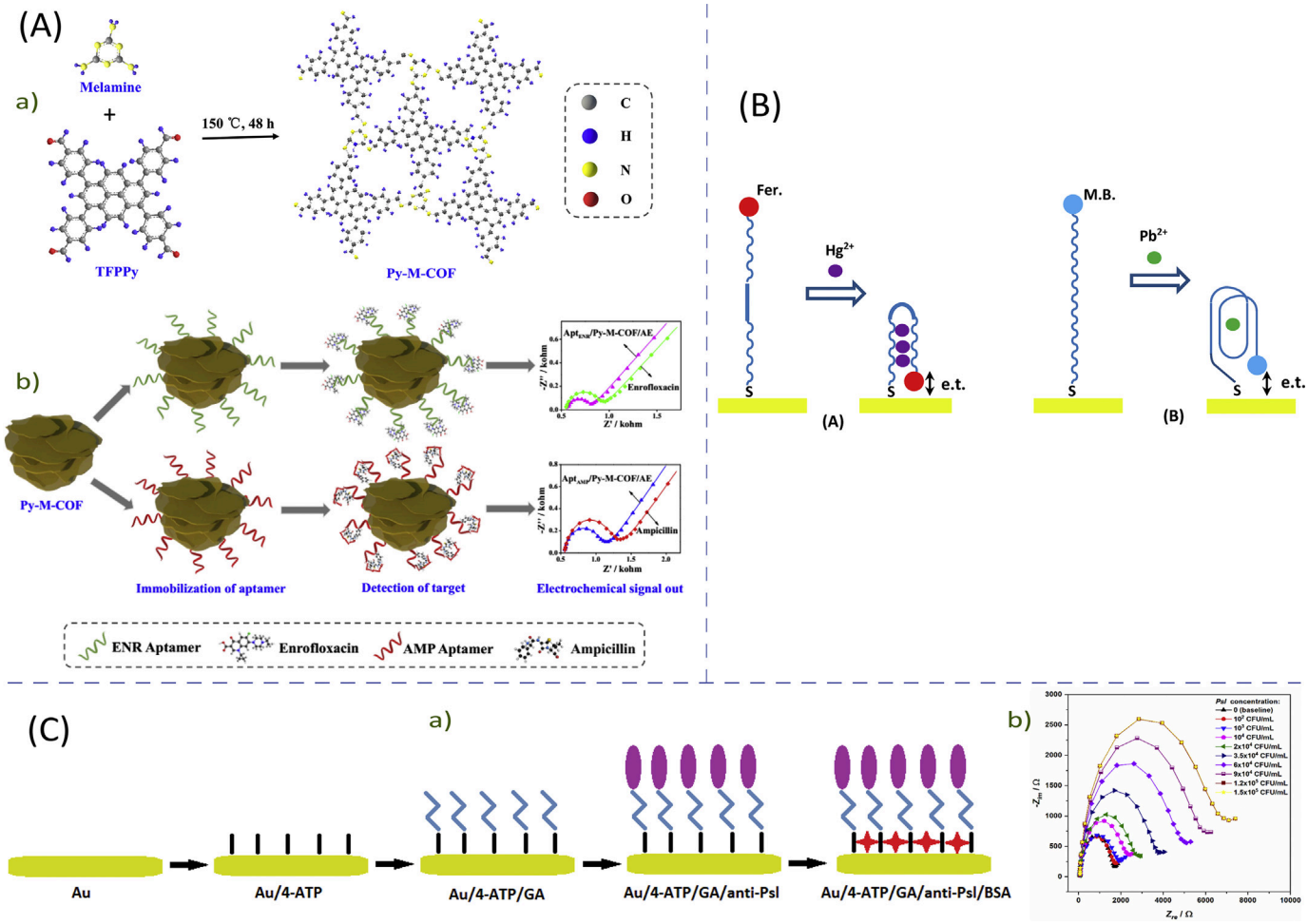


Fig. 5. (A) a) Schematic diagram of the synthesis of Py-M-COF and b) the electrochemical detecting procedure for enrofloxacin (ENR) and ampicillin (AMP) by the Py-M—COF—based aptasensors [173]; (B) Schematic illustration of electrochemical detection for Hg^{2+} and Pb^{2+} using redox-labelled aptamers [174]; (C) Fabrication of biosensor and the linearity response on different concentrations of *Psl* [175].

printed gold electrodes immobilised with aptamers by thiol groups [174]. These two aptamers were labelled with ferrocene for Hg^{2+} and methylene blue for Pb^{2+}. In the presence of target ions, the aptamers would chelate the targets and changed the conformation to a hairpin structure for anti-Hg aptamer and G-quadruplex for anti-Pb aptamer (Fig. 5B). After that, the closer redox labels to the electrode led to the increase of electron transfer which was recorded by the electrochemical current. The LOD for Hg^{2+} and Pb^{2+} was as low as 0.1 ng mL^{-1}. In another study, Cebula et al. developed an antibody-modified gold electrode sensor for the detection of *Pseudomonas Syringae pv. Lachrymans* (*Psl*) [175]. In the assay, 4-aminothiophenol (4-ATP), glutaraldehyde (GA), and anti-Psl antibodies were successively immobilised on the gold disc electrode with bovine serum albumin (BSA) as free-sites blocking (Fig. 5C). The detection is achieved within 10 min after easy preparation. The linear concentration of *Psl* ranges from 1×10^3 to 1.2×10^5 CFU mL^{-1} with a LOD of CFU mL^{-1}.

The analytical performances of the electrochemical sensors for various analytes are provided in Supplementary Data (Table S2).

4.3. Microfluidic sensors

Microfluidic sensors are highly miniaturised and integrated with the advantage of excellent selectivity, rapid analysis, low cost, tiny reagent consumption [176,177], which has been extensively applied in the fields of chemistry, materials, biology, medical diagnosis, and drug analysis. Recently, microfluidic sensors have been used for environmental pollutants analysis, such as antibiotics, heavy metals, antimicrobial resistance genes, and pathogens. For instance, Chang et al. developed an integrated microfluidic system for the automatic identification of vancomycin-resistant gene (*vanA*) in live bacteria from clinical samples [178]. Initially, the dead and live bacteria were treated with ethidium monoazide (EMA) for the chemical lysis under low temperature and nucleotide probe hybridization (Fig. 6A). Then, the target DNA was attached to the nucleotide probes and isolated by the magnetic beads, following by a fluorescent LAMP assay within less than 1 h and showing a LOD of 10 CFU for *vanA* from live *Enterococcus*.

Besides, Jasim et al. developed an impedance microfluidic sensor with three microchannels to simultaneously detect three *Salmonella* serogroups [179]. Each channel included a focusing region to direct the *Salmonella* cells in the centreline, toward the sensing region (Fig. 6B). The positive di-electrophoresis force was used for highly concentrated samples. Ten pairs of fingers consisted of an interdigitated electrode (IDE) constituted the sensing region. The antibodies of three *Salmonella* types (B, D and E) were immobilised on the detection electrodes for each channel. After adding type B spiked *Salmonella* samples in the inlet region, they were introduced through the focusing region to the sensing region. When the target antigen was bound to the antibody, the impedance

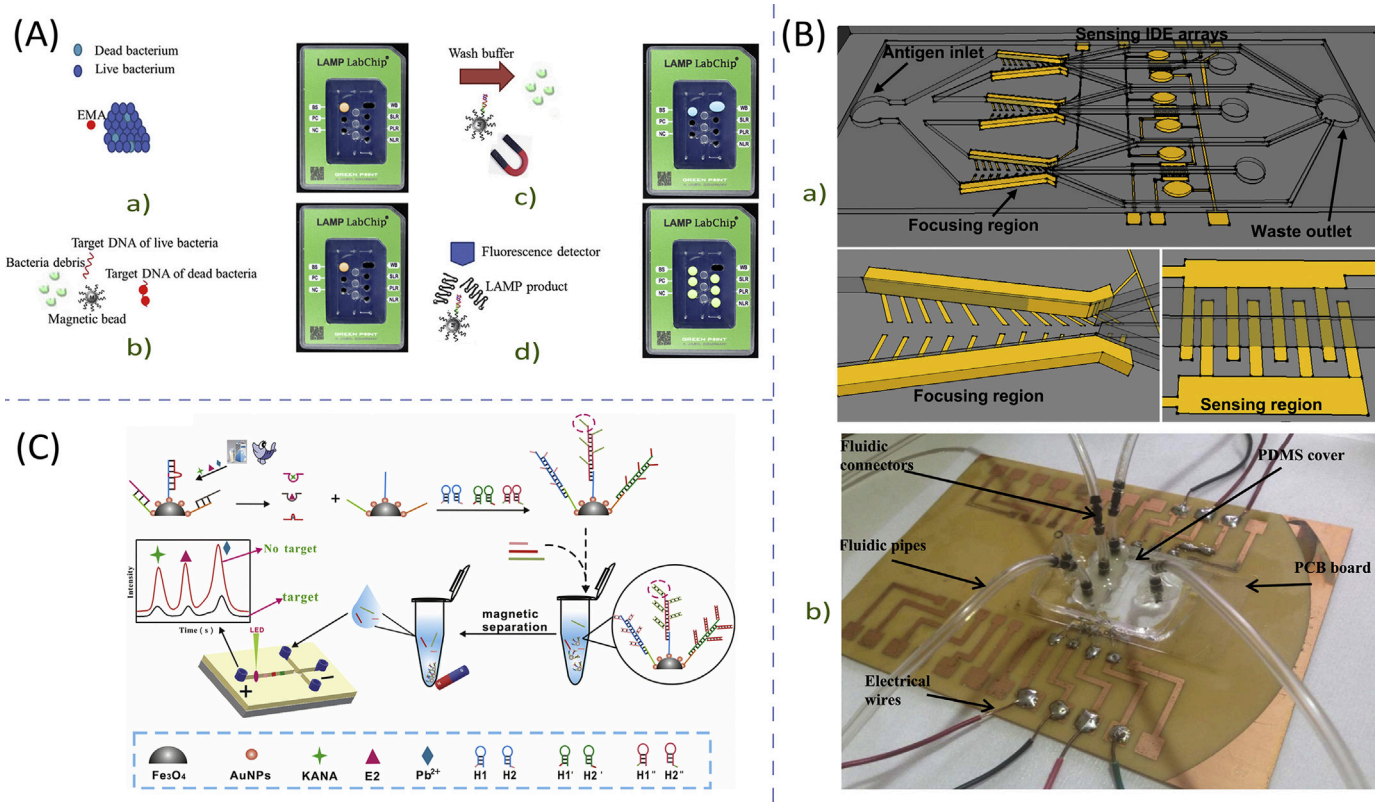


Fig. 6. (A) The schematic detecting procedure of the integrated microfluidic system for *vanA* [178]. (B) a) Schematic illustration of the impedance-based biosensor for *Salmonella*, enlarged display of the focusing electrode and detection electrodes; b) Physical picture of the biosensor on a PCB board [179]. (C) Schematic illustration of the fabrication of biosensor and the detecting mechanism for different types of analytes (kanamycin, 17β-estradiol and Pb^{2+}) based on the microfluidic system [35].

change was recorded. This sensor can recognise the single *Salmonella* serotype B or the mixed two *Salmonella* serotypes B, and D in 40 min with of the LOD of 7 cells mL^{-1}. Further, *Salmonella* can also be selectively detected in the presence of other pathogens such as *Escherichia coli* O157: H7 and the design of this device provides a non-enrichment step for many samples.

Other studies also demonstrated the possibility of detecting the different type of contaminants and environmental stressors simultaneously. For example, Chen et al. reported a multi-analysis method based on a microfluidic chip (MC) which was coupled with magnetic encoded aptamer probes to simultaneously detect kanamycin (KANA), 17β-estradiol (E2), and Pb^{2+} [35]. Multiple targets were firstly captured by the gold modified magnetic bead (MB) probes labelled with aptamers and then the single-stranded primers were generated initiating a multibranched hybridization chain reaction (mHCR) (Fig. 6C). Finally, the arms of the mHCR products were hybridised with three different length cDNAs to produce three types of multibranched DNA nanostructures. They were then separated by the magnetic bead (MB). The reduced signals of cDNAs in the supernatant reflected the qualification of targets. The LOD for KANA, E2 and Pb^{2+} were 1.76×10^{-4} nM, 1.18×10^{-4} nM and 1.29×10^{-4} nM, respectively. In this assay, different targets corresponded to their separated signal tags, decreasing the massive cDNAs for one target. Also, the MB probes can be magnetically separated to eliminate interference in the complex. Further, the MC platform can be reused for more than 4000 samples. Such assay can detect various chemical contaminants by changing the aptamers and can also be applied in the detection of environmental samples.

Paper-based microfluidic sensors is an attractive technology which is growing fast since the Whitesides' group reported in 2007 the first paper analytical device [180]. Paper-based microfluidic sensors generally use printing conductive materials as the substrate which can hold 0.1–100 mL of liquids in the millimetre dimensions channel. Each layer of the assays can be achieved by stacked, folded, and laminated to fabricate the simple device with easy operation. Recently, paper-based microfluidic sensors have been popular in the analytes of the environment due to their merits of convenient carriage, rapid detection and low cost. A simple paper device for antibiotic detection can be fabricated by the paper with specific materials for colourimetric sensors. For example, Ha et al. utilised the AuNPs developed a wax printed paper device for kanamycin detection in milk [181]. The AuNPs aggregated and changed colour from red to blue in the presence of kanamycin with the addition of NaCl within 30 min (Fig. 7A). By modifying the materials and/or chemicals integrated on the paper, the paper device can be used for different analytes [181,182]. For instance, Devadhasan and Kim [183] used a chemically patterned microfluidic paper-based analytical device (C-μPAD) for the detection of heavy metals. The heavy metals are immobilised with silane compounds coupled with amine (NH2), carboxyl (COOH), and thiol (SH) termination (Fig. 7B). These function groups were then covalently coupled to three chromogenic reagents which can distinctly react with Ni^{2+}, Cr^{6+} and Hg^{2+} within 1 min. Using the single-plex platform, they achieved a LOD as low as 0.24 ppm, 0.18 ppm, and 0.19 ppm for Ni^{2+}, Cr^{6+} and Hg^{2+} respectively. Such portable device is a good example that can help further rapid detection method for multiple analytes in the environment.

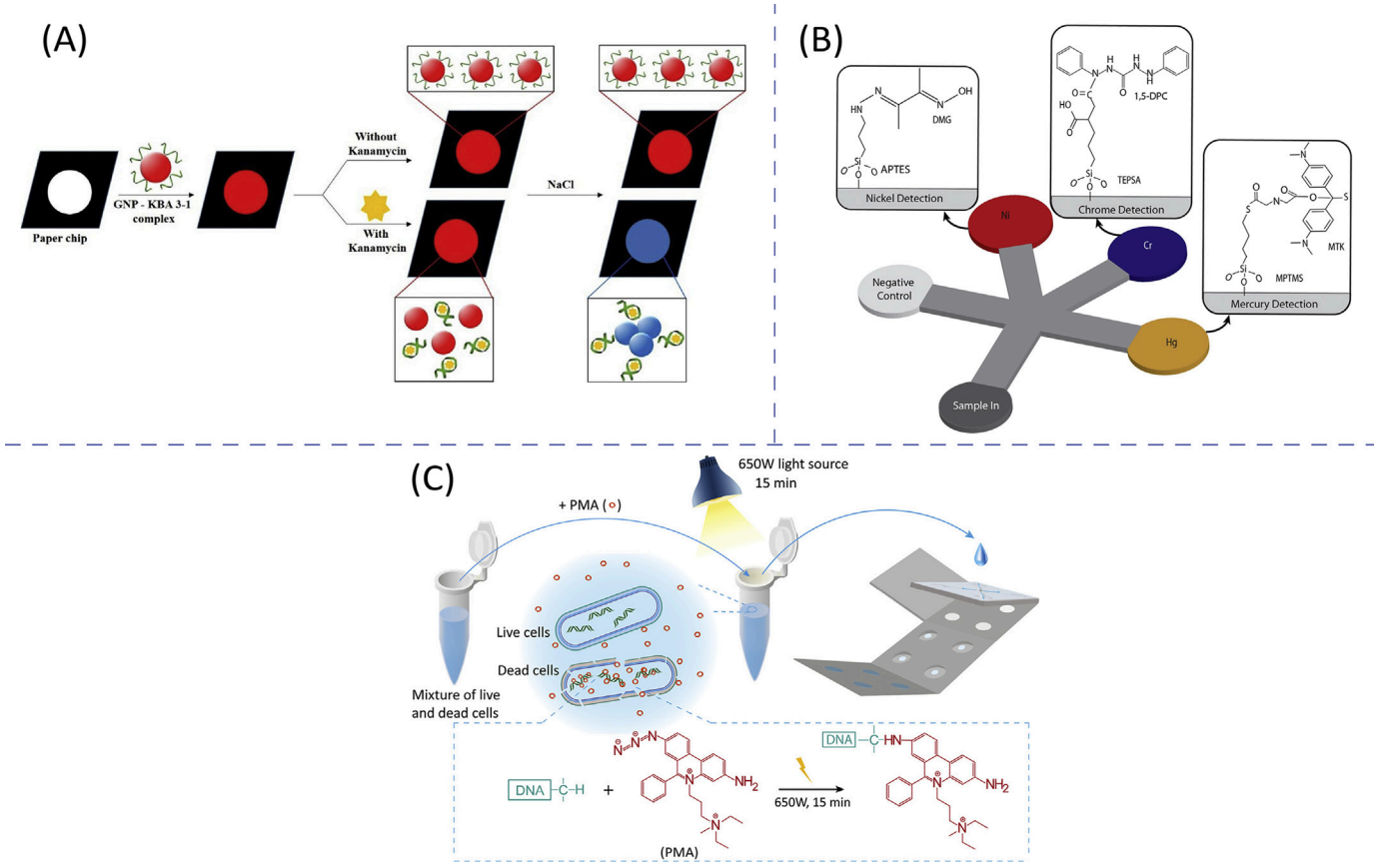


Fig. 7. (A) Schematic illustration of the fabrication of paper chip-based colourimetric sensor for kanamycin detection [181]. (B) Schematic of illustration of various heavy metal detection on the multiplex C-μPAD [183]. (C) Schematic illustration of sample preparation and the detection for pathogens on the origami paper microdevice [184].

As for the detection of pathogens, a common strategy is to combine LAMP with paper-based microfluidic biosensors [100,185]. One simple paper origami colourimetric device has been developed for the detection of pathogens [184]. This all-in-one origami paper device integrated the DNA purification, LAMP and on-site detection (Fig. 7C). Microchannel networks and chambers on the origami paper were firstly created which were then coated with hydrophobic polydimethylsiloxane prepolymer for stabilization of structures and providing fluid barriers. The specific nucleic acid amplification testing was accomplished through the integrating of cell splitting and purification, and target wicking, reaction, and dye pads folding alternatively on the paper microdevice. To obtain the DNA only from the live cells for amplification, the dead cells were penetrated by propidium monoazide (PMA) to be covalently bound to their DNA. Subsequently, chitosan was utilised for the purification of DNA into the microdevice through electrostatic capture, and the methylene blue was introduced for the colourimetric detection of LAMP amplicons. This paper microdevice can detect various pathogens such as *Escherichia coli* O157: H7 and *Salmonella* spp. with the LOD of 25 CFU mL^{-1} under the amplification of 30 min and the colourimetric detection of 10 min. Methylene blue is generally used in the electrochemical detection while it was used for colourimetric detection here which manifests that some chemicals can be mutually utilised in different assays.

A range of microfluidic sensors for various analytes detection are provided in the Supplementary Data (Table S3).

In summary, the colourimetric sensors are cost-effective due to the no requirement of additional instruments which can be recognised by naked eyes. They are the most suitable for on-site detection. However, colourimetric sensors remain the shortage of relatively low sensitivity and selectivity compared to fluorescence sensors which possess generally lower LOD values of analytes. As for electrochemical sensors, they have exclusive advantages of rapid response, low cost, and small size which are easy to be integrated with other devices and are executable for the real sample detection. Microfluidic technology develops commonly combined with electrochemistry, fluorescence, colourimetric, SPR, paper-based device, etc. They are usually applied for multiple targets, especially the paper-based microfluidic device, a highly promising technique, which is portable for on-site detection owing to their simple operation and multiple-analytes detection.

5. Strategy for the rapid detection and monitoring during remediation of contaminated sites

Even though the rapid methods for various analytes and the remediation measures for contaminated soil have been developed for many years, the prevalence of AMR has largely been ignored until now. There is an urgent need for the development of technologies that allow rapid assessment on site to inform bioremediation appraisal, monitoring and end point.

Further, even though there is the possibility of existing assays for the monitoring of various analytes in the environment due to the same working principle [217,218], each method has its own focus on different targets due to their specific characteristics. Especially, petroleum hydrocarbons are seldom monitored by the current rapid methods due to the specific characteristic of hydrocarbons such as the weak interaction between hydrocarbons and other materials [170]. Development of rapid methods for those

analytes is required for better monitoring of the environmental contaminants.

5.1. Insights on the suggestion for the development of rapid detection methods

Based on the working mechanism, implementation of devices with the capability for multiplexed detection of analytes (i.e. antibiotics, heavy metals, hydrocarbons, etc.) is the ultimate goal of the rapid methods. Of course, the microfluidic technology especially paper-based microfluidic sensors which can be integrated with different transducers (e.g. colourimetric, fluorescent, electrochemical, etc.) is the promising assay in the future.

The EIS based sensors can monitor various analytes even the hydrocarbons from oil or diesel [186,187]. Researches have also developed the EIS sensors for "green" on-site metal detection directly in the soil, indicating the potential for field application [188]. Therefore, the future development of rapid methods can be integrated with a wide range of sensors which can target more analytes, especially for the ARGs and hydrocarbons. There is also a need for the integrations of multidisciplinary solutions, involving engineering approach with nanotechnology and novel nanomaterial to improve the sensitivity and selectivity.

For the detection of solid samples, the targets are in the solvent after pre-treatment of the solids and therefore the current state-of-art sensor technology is easy to be adaptable for soil samples. Besides, studies focus on the solid samples like manure, sludge and soil have been inadequate due to the tedious pre-treatment. This hence needs to incorporate the development of sample processing technique of solid samples and detection methods. Even though there are some studies on solid samples such as food, they still need the pre-treatment for analytes extraction [189,190]. The development of rapid methods should focus on both the detection of specific targets and the pre-treatment protocol with integration for the detection of complex environmental samples.

5.2. Strategies for soil remediation from the perspective of AMR situation

From the current developed various rapid methods, we have no vested standard to assess the quality of one method as the researchers only provide their detecting parameters like the dynamic range and LOD. When investigating the situation of farmland or contaminated land, studying as many factors as possible to obtain an overall survey is essential, especially during the application of bioremediation. We know that the microorganisms and pollutants like antibiotics, heavy metals, hydrocarbons, exert some effects on the AMR in the soil. The monitoring of extensive indicators will provide us with a synthetic understanding of the current situation. This is also the motivation to develop the future integrated platform aiming for multiple analytes.

On the other hand, more attention should be paid on the issue of AMR in soil, especially when developing the remediation strategy. Soil amendments (i.e. manure, sludge, biosolids) containing trace contaminants such as heavy metals, microorganisms and ARGs, are expected to be carefully considered for the remediation. A comprehensive understanding of the polluted soil can be achieved by the rapid detection of various analytes and the soil amendments. Ultimately, the control of pathways for pollutant source into the soil is the most essential factor for the environmental governance and ecological health.

6. Conclusion and perspectives

The prevalence of AMR in contaminated soils and/or soil undergoing bioremediation has been largely overlooked until now. Recent recognition of the potential risk associated with antibiotic-resistant bacteria and genes is calling for further investigation and also prompting for the need of rapid measurement tools to inform risk and sustainable bioremediation strategies. Thereby there is a need for integrated assays allowing simultaneous field-based detection of various analytes including antibiotic residues, heavy metals, petroleum hydrocarbons, ARGs, and pathogenic bacteria. Key criteria for the selection and the development of rapid methods include the selection of appropriate specific receptors, surface coating, and proper amplification strategy to ensure a highly sensitive and specific assay. A range of engineering method can be implemented the assay for a portable device for rapid on-site detection of soil samples. The combination of optical or electrochemical sensors with the microfluidic sensors is a promising choice for the simultaneous detection of complex biological and chemical mixture in soils. Those rapid method will provide an immediate monitoring result of contaminated soil to inform risk assessment and remediation strategy in a timely manner and minimise the risk of propagating AMR in the environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trac.2021.116203.

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Zhou, Cailing; Pan, Yuwei; Ge, Shifu; Coulon, Frederic; Yang, Zhugen

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