Theragnostic application of nanoparticle and CRISPR against food-borne multi-drug resistant pathogens

Rahul Bhattacharjee [a,1], Aditya Nandi [a,1], Priya Mitra [a], Koustav Saha [a], Paritosh Patel [a], Ealisha Jha [a], Pritam Kumar Panda [b,**], Sushil Kumar Singh [c], Ateet Dutt [d], Yogendra Kumar Mishra [e,***], Suresh K. Verma [a,b,*], Mrutyunjay Suar [a,****]

[a] KIIT School of Biotechnology, Kalinga Institute of Industrial Technology (KIIT-DU), Bhubaneswar, Odisha, India
[b] Condensed Matter Theory Group, Materials Theory Division, Department of Physics and Astronomy, Uppsala University, Box 516, SE-751 20, Uppsala, Sweden
[c] DBT- NECAB, Department of Agricultural Biotechnology, Assam Agriculture University, Jorhat, 785013, Assam, India
[d] Instituto de Investigaciones en Materiales, UNAM, CDMX, Mexico
[e] Mads Clausen Institute, NanoSYD, University of Southern Denmark, Alsion 2, DK-6400, Sønderborg, Denmark

ARTICLE INFO

ABSTRACT

Foodborne infection is one of the leading sources of infections spreading across the world. Foodborne pathogens are recognized as multidrug-resistant (MDR) pathogens posing a significant problem in the food industry and healthy consumers resulting in enhanced economic burden, and nosocomial infections. The continued search for enhanced microbial detection tools has piqued the interest of the CRISPR-Cas system and Nanoparticles. CRISPR-Cas system is present in the bacterial genome of some prokaryotes and is repurposed as a theragnostic tool against MDR pathogens. Nanoparticles and composites have also emerged as an efficient tool in theragnostic applications against MDR pathogens. The diagnostic limitations of the CRISPR-Cas system are believed to be overcome by a synergistic combination of the nanoparticles system and CRISPR-Cas using nanoparticles as vehicles. In this review, we have discussed the diagnostic application of CRISPR-Cas technologies along with their potential usage in applications like phage resistance, phage vaccination, strain typing, genome editing, and antimicrobial. we have also elucidated the antimicrobial and detection role of nanoparticles against foodborne MDR pathogens. Moreover, the novel combinatorial approach of CRISPR-Cas and nanoparticles for their synergistic effects in pathogen clearance and drug delivery vehicles has also been discussed.

1. Introduction

Foodborne MDR pathogens are primarily recognized as one of the main causes of infectious diseases worldwide considering their presence in varieties of food as an important carrier for transmission [1,2]. According to World Health Organization (WHO), foodborne diseases are caused by MDR pathogens which include *Salmonella* spp.*, Staphylococcus aureus, Listeria monocytogenes, Shigella* spp.*, Clostridium botulinum, Escherichia coli* O157:H7 [3]. The WHO estimates around 600 million cases due to foodborne diseases causing approximately 42,00,00 deaths thereby resulting in the loss of 33 million healthy life years annually [3]. The rapid increase in foodborne diseases, coupled with the emergence and re-emergence of foodborne pathogens, have put food safety at high risk for public health [4]. These foodborne pathogen accounts for almost two-thirds of the human foodborne diseases worldwide with a considerable amount of risk in the developed countries; which thereby necessitates the development of novel therapies for combating this MDR pathogen [4–6]. The WHO has reported the rapid rise in infections was mostly due to methicillin-resistant *Staphylococcus aureus*, cephalosporin, and fluoroquinolone-resistant *Escherichia coli*, fluoroquinolone-resistant

Campylobacter species, and streptomycin, gentamicin, tetracycline-resistant *Salmonella* species [4,7]. Food-borne pathogens, being diverse, accounts for most of the food-borne diseases creating major health problems in human. It is more at risk for infants, elderly people and immunocompromised individuals through ingestion of contaminated food, causing diarrhoea, stomach ache and vomiting as symptoms post-manifestation [8,9]. Food contamination of meat, eggs and milk products is major an issue which occurs at any stage of the food chain and requires to be dealt to prevent outbreaks like 2017(reported by EFSA and CDC) [10] (see Tables 1 and 2).

The MDR pathogens which causes the diseases have different molecular strategies to overcome the effect of the antimicrobial drugs induced for their killing. Some of these mechanism includes the resistant to the inhibition of the nucleotide synthesis, disruption of cell membrane, and reduced protein synthesis caused by the induced antimicrobial drugs. Another antimicrobial resistant (AMR) mechanism exhibited by these MDR pathogens includes fluoroquinolone resistance due to mutations in DNA gyrase and topoisomerase IV genes. Similarly, tetracycline resistance mechanisms include transport-based mechanisms by protecting ribosomal binding site of tetracycline via RNA-binding proteins (RBP) in MDR pathogens [11]. Even though traditional microbiological approaches for detection and mitigation of MDR related problems are considered to be gold standard methods in food industry, yet they poses rate limitation [12]. The rapid detection and identification of pathogens is crucial in the theragnostic applications. Which poses a varied widespread opportunity for utilizing CRISPR-Cas complex techniques for regulating food safety and environmental monitoring. The CRISPR-Cas complex serves as an excellent defense system for adaptive immune responsive tool in prokaryotes, regardless of archaea (~90%)) or bacteria (~40%). It is useful to identify reoccurring infections by integrating short sequences of invading genomes, known as spacer sequence [13]. Most bacterial species possess an inherent noble CRISPR-Cas9 system, which makes it a promising detection tool over other traditional approaches for uncovering of food pathogens. Till date, CRISPR-Cas systems can be classified into two main category based on the occurrence of effector Cas protein, which further can be segregated into six different types and subtypes [14–16]. Despite of having high level of diversification, CRISPR-Cas9 works in a similar fashion based on sequence specificity by recognizing and cleaving foreign DNA/RNA. The mechanism can be staged in to three major events; i) integration into CRISPR array or spacer acquisition, ii) crRNA maturation and iii) target interference as illustrated in Fig. 1 [13]. Besides, the application of CRISPR-Cas9 system (pCasSA) extends beyond genome editing; that includes high resolution typing of pathogens, manipulation of MDR genes, designing vaccine of starter culture against phages. This further pushes the boundary of deciphering new mechanisms of different CRISPR-Cas systems with new cas genes and enzymatic activities. The major focus is to use the CRISPR system as an antimicrobial and detection tool for pathogen clearance. CRISPR-Cas9 editing system has also been used to successfully demonstrate the sequence specific removal of AMR gene bearing plasmid thus making the bacteria susceptible to antibiotics. Moreover, CRISPR-Cas system presents in the genome of the pathogens aids as a detection marker for food borne pathogen. The major issue with CRISPR-Cas system is its delivery. The use of novel non-viral delivery strategies using nanoparticle aids to transport the CRISPR-Cas cargoes to the desired bacterial strain enhancing the CRISPR-Cas efficiency [17].

Table 1
Application of NPs as an antimicrobial and detection tool.

NPs	Characterization	Type of preclinical study	Pathogen	Result	References
Ag-NPs	Size-75nm(spherical) and 8–20 nm (triangular)	RCT	*E.Coli* (Gm-ve*)*, *Staphylococcus aureus(*Gm + ve*)*, *Salmonella typhi* (Gm-ve)	The highest antimicrobial activity of Ag-NPs was shown against E.*coli* while S.*typhi* showed moderate activity and it was low for S.*aureus*.	[151]
Ag-NPs	Size-8-15nm	RCT	*E.coli* O157:H7 (Gm-ve), *Listeria monocytogenes* (Gm + ve*)*	The material containing Ag-NPs exerted greater antimicrobial activity against L.*monocytogenes* than E.coli because of greater cell wall thickness of Gram-positive bacteria.	[27]
ZnO-NPs	Size-8nm	*In vitro*	E.*coli*(Gm-ve) and S.*aureus*(Gm + ve)	The material containing ZnO-NPs inhibited the growth of both E. *coli* and S.*aureus*	[152]
ZnO-NPs	Surface-volume ratio	*In vitro*	E.*coli*(Gm-ve) and S.*aureus*(Gm + ve)	Antimicrobial activity against both E.coli and S.aureus increased with increase in surface to volume ratio with decrease in particles size of ZnO-NPs.	[152]
Nano-Colloidal Silver	MIC/MBC conc.	*In vitro*	*E.coli*(Gm-ve), S.*aureus*(Gm + ve), *Listeria monocytogenes (Gm-ve), Salmonella enterica* (Gm-ve)	The MIC values showed average range of 7–25 ppm whereas the MBC values for all the bacteria were above 100 ppm which showed good inhibitory effect but poor bactericidal effect of Nano-Colloidal Silver	[108]
Graphene nanosheets	Molecular dynamics stimulation	*In vitro*	E.*coli* (Gm-ve)	Graphene nanosheets induce degradation of the cell membrane with the extraction of phospholipid molecules from the membrane leading to bacterial inactivation.	[153]
Pd-Nps	Size - 2 nm	*In vitro*	E.*coli* (Gm-ve), S.*aureus* (Gm + ve)	Greater antimicrobial activity of Pd-NPs was observed ast concentrations as low as 2.5 nm in case of Gm + ve S.*aureus* as compared to Gm-ve E.*coli*	[154]
Prismatic shaped Y_2O_3-NPs	Shape	*In vitro*	S.*aureus* (Gm + ve)	Greater antimicrobial activity was shown against S.*aureus* which is mainly influenced by the shape of the Y_2O_3-NPs occurring due to direct interaction between prismatic shaped Y_2O_3-NPs and bacterial cell membrane leading to breakage of the cell membrane.	[155]
Silver NPs and streptavidin-coated magnetic beads	Size – 20 nm	*In vitro*	S.*aureus (Gm + ve)*	Detection sensitivity of $10–10^6$ CFU/mL, detected by an electrochemical immunosensor through sandwich immunoassay on biotinylated primary anti S.*aureus* aptamer and secondary anti S.*aureus* aptamer	[156]
Mesosporous TiO_2 coated magnetic NP	Size - 200 nm	*In vitro*	E.*coli* (Gm-ve) *and* S.*aureus*(Gm + ve)	Detection sensitivity of 10–2000 CFU/mL, detected through fluorescent imaging with aptamer based recognition.	[157]
Gold NP	Size – 20 nm	*In vitro*	S.*typhimurium* (Gm-ve)	High level of sensitivity and specificity for detection of S.*typhimurium* in pork meat detected by LSPR signal sensing with aptamer based recognition	[158]

Table 2
Thero-diganostic Application of CRISPR-Cas system.

CRISPR Cas	Type of preclinical study	Pathogen	Result	References
Cas9	In vivo	*Staphylococcus aureus*	Phagemid targetting methicilin resistance gene mec^A to selectively eradicate virulent and avirulent S.*aureus*	[77]
casABCDE and cas3	*In vitro*	*Escherichia coli, Salmonella enterica*	Examined selective removal of individual strains in pure and mixed cultures	[73]
Cas9	*In vivo* and *In vitro*	Enterohemorrhagic *E. coli* (EHEC), *E. coli*	Phagemid delivery resulted in 20 fold reduction in EHEC in case of *in vitro* and in case of *in vivo* it showed increase in survival rates in Galleria mellonella	[75]
Cas13a	*In vitro*	*Staphylococcus aureus*	A novel CRISPR-Cas13a based bacterial detection method, CCB detection tool successfully detects the target gDNA with a dynamic selction range revealing enhanced selectivity of S.*aureus* by rapid detection assay.	[17]
dCas9	*In vitro*	*Escherichia coli*	Genome wide screening of CRISPR-dCas9 by utilizing sgRNAs targeting random E.coli chromosome locations, utilizing CRISPRi during gene essentiality prediction in the E.coli genome thereby encompassing the full phage cycle.	[50]
Cas13a	*In vitro*	*Salmonella enteritidis*	A detection technique called APC-Cas utilizes a combination of allosteric probes based on nucleic acids and CRISPR-Cas13a to detect low single cells of S.*enteritidis* with low cross reactivity with other bacteria.	[59]

In recent time, nanotechnology has been revolutionizing the food industry with its various showcased applications in different dimensions such as use of low-cost nano-sensors for detection, quantification of pathogens, alteration of food composition, food deterioration control, and use of target delivery of nutrients or bioactive molecules through nano-encapsulation [18,19]. Nanoparticles has been a promising candidate in food industry owing to its widespread application against MDR pathogens due to its peculiarity in variation in attainable size and shape that can penetrates directly to the bacterial cell [20,21]. The underlying mechanism of antimicrobial effects of NPs has been described to be mediated through different ways such as: i) generation of ROS [22], ii) disruption of bacterial cell membrane [23], iii) penetration of bacterial cell membrane, iv) influencing the physiological process through interactions with DNA and proteins [24,25]. Nanoparticles has been portrayed as vector for the CRISPR-Cas delivery and a potential detection tool for pathogens [26]. Concerning the potential usage and increasing popularity of nanotechnology [27] and CRISPR-Cas technique, it is the need of hour to review in detail about the two strategies and the potential combinatorial approach for a higher output in food industry in respect of efficiency and speed.

The reviews discuss the application of nanoparticle and CRIPSR-Cas to combat food borne pathogens in the management of their manifested infections. Herein, we have not only discussed the theragnostic application of CRISPR-Cas system and NPs as detection tools and antimicrobial against foodborne pathogens but also highlighted a novel combinatorial approach to their synergy against foodborne pathogens. Moreover, we have elucidated the application of the CRISPR-Cas system as phage vaccination, plasmid resistance, and genetic editing tool. This review will pave the way for researchers to revolutionize personalized medicine through the use of nanomedicine and CRISPR-Cas system both alone as well as in synergy. These will advocate the early onset diagnosis and selective killing of food borne pathogens.

2. CRISPR cas complex as a detection tool against food pathogens

The seriousness of food-borne infections caused by MDR pathogens or their toxins necessitates the rapid development of a precise and sensitive technology for combating these pathogens [17]. Conventional microbiological methods are considered as the gold standard approach because of their reliability and accuracy. However, their limitations of prolonged, laborious, and destructive mode of application impose issues for their accuracy and long term usage in the food industry [12]. Similarly, the other famous approaches which are based on immunological methods and their combination also have limited utility for food pathogen identification [28]. Additionally, the other traditional pathogen detection techniques which are biosensor-based and nucleic acid-based are used as

an effective tools, despite their limited application in the food sector [28]. Concerning to these limitations, researchers are always being in search of new, advanced and powerful techniques for pathogen diagnostics. One of the most recent technique is the use of CRISPRs-Cas systems and nanotechnological inventions to design new diagnostics methods.

Most of the sequenced bacterial genomes (45%) CRISPRs-Cas systems act as an adaptive immunity for them [29]. These CRISPR/Cas9 system (pCasSA) has been designed as a powerful tool to inhibit transcription in gene knockdown strategies, genome-wide screening, and genomic modifications through mutations. The techniques using inhibition of transcription through CRISPR/Cas9 system for gene-editing techniques has progressed significantly in recent years [30]. The utilization of Cas proteins having variable functionality has resulted in more sensitive and reliable nucleic acid detection systems. The extremely variable spacer sequences residing within the CRISPR array are separated from one another by invariant direct repeat sequences of CRISPR-Cas-based detection techniques [31]. Recent studies have showed the potential of using these new CRISPR-Cas technologies for cost-effective pathogen detection tools as illustrated in Fig. 2 [32]. Some of the listed one includes specific to the some MDR pathogens like *Staphylococcus aureus, Salmonella typhi* etc. as described; *Staphylococcus aureus* is a MDR food borne pathogen belonging to the *Micrococcaceae* family that causes several deadly infectious disorders with a high fatality rate [33]. It is present in humans as a natural resident exhibiting colonization effects in 30–50% of healthy persons while 10–20% of the human population are persistently colonized by MDR strains like methicillin-sensitive and methicillin-resistant strains(MRSA) [34–36]. The colonization establish with the phenomenon of biofilm formation which makes them resistant against the immunological action in body, thus making the infection more challenging. Moreover, further dispersal of bacterial cells from the biofilm during infection leads to their transmission to other body secondary sites [37]. Though vancomycin (a glycopeptide), daptomycin (a lipopeptide), and linezolid (an oxazolidinone) are deemed effective against MRSA infection, its long-term usage renders the strains resistant to deadly conditions that needs to be detected and diagnosed for treatment [38]. Although genetic alternation of *S. aureus* is tedious and laborious, it is an effective tool for investigation of pathogens physiology. A bacterial sensing technique called CCB-Detection has been developed, by utilizing advantage of the crRNA-based programmability of the CRISPR-Cas13a system and its RNase activity to target RNA recognition against *Staphylococcus aureus* [17]. CCB-Detection is capable enough to target gDNA successfully with a dynamic detection range (10^0–10^7 CFU/mL) for food samples containing known and unknown concentrations of bacteria (spiked and non-spiked). The technique further reveals enhanced selectivity for *S. aureus* by rapid detection assay [17].
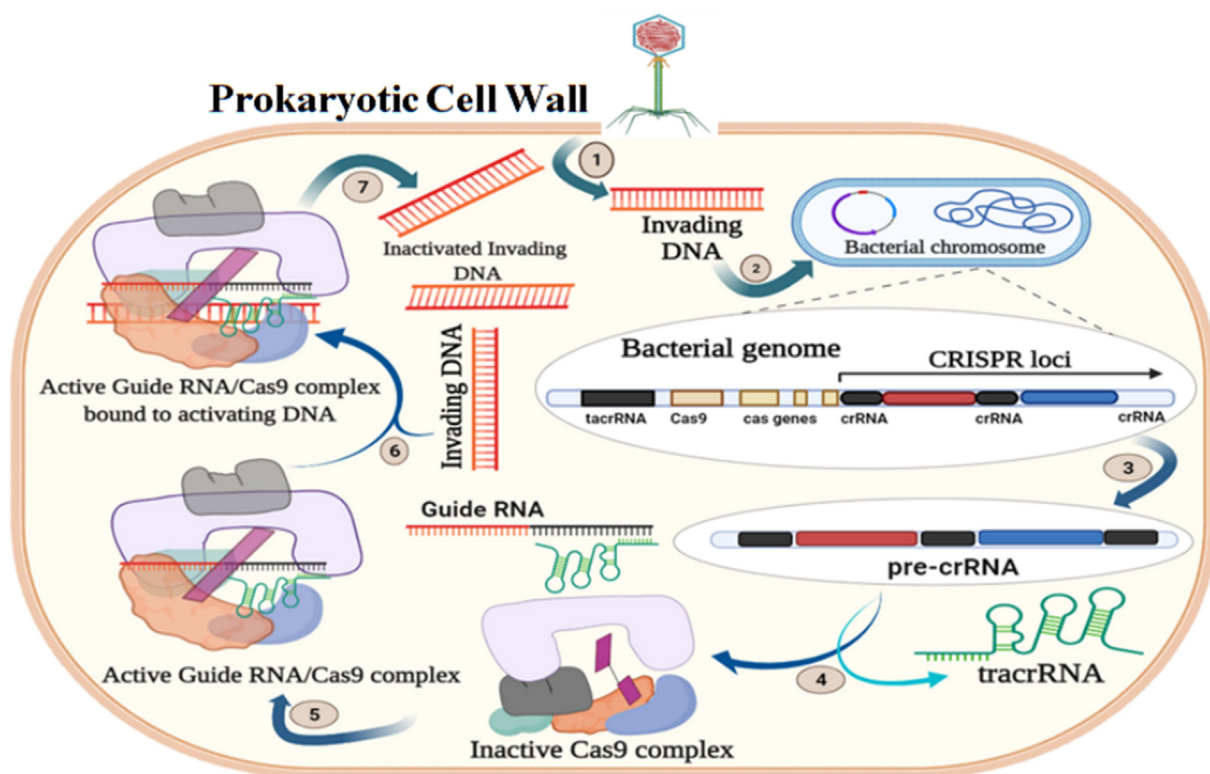
Fig. 1. Illustrative representation of CRISPR-Cas general mechanism. **1**. Invasion of DNA where a foreign DNA from a plasmid invades the cell, **2**. Incorporation of the DNA fragments from the invading DNA into the CRISPR locus as spacers, **3**. pre-cRNA transcription occurs where the cell constitutively transcribes a spacer group, **4**. Formation of guide RNA takes place where transactivating (tracrRNA) base pairs with the CRISPR repeat sequences on the pre-crRNA, **5**. Activation of Cas9 protein takes place where inactivated Cas9 protein complex binds to the guide RNA and thus gets activated, **6**. The activated guide RNA/Cas9 protein complex binds with the target DNA, **7**. Inactivation of the target DNA which is cleaved by the Cas9 protein.

Escherichia coli O157:H7 is one of the most harmful foodborne pathogenic bacteria, causing an estimated 73,000 cases of infection in USA annually [39]. *Escherichia coli* belongs to the family of Gram-negative bacteria found as both pathogens and commensals in mammalian intestines and cause infections of urinary tract, neonatal meningitis, sepsis, pneumonia, surgical site infections through colonization [40]. The clonal dissemination of intestinal pathogenic *E. coli O157:H7*, primarily lead to contamination of food products [41]. The infection caused by food like diarrhoea in children is not only extremely contagious, but their transmission is unidentifiable [42]. Therefore, detecting the most common pathogens in foods and environmental sources would aid in better treatment. Standard culture-based approaches, qPCR, ELISA, have been conventionally applied to identify *E. coli* O157:H7 in the previous decades but are time-consuming, complex processes, and have low sensitivity [43–45]. Researchers have used several methodologies to detect *E. coli* O157:H7, including fluorescence, chemi-luminescence, and electrochemical detection in combination with various biosensors for quick analysis and great sensitivity [46–48]. The CRISPR Cas system has arisen as a new tool for the detection and antimicrobial of *E. coli*, supplementing the limits of conventional approaches due to its remarkable selectivity and genetic editing properties [49]. A genome-wide CRISPR-dCas9 screening by utilizing sgRNAs targeting random *E. coli* chromosome locations has been proved as an efficient method for diagnosis that utilizes CRISPR for the prediction of essential genes in the *E. coli* genome during *in-vitro* growth of functional capsids, encompassing the full phage cycle. Furthermore, the broad usage of CRISPR-dCas9 screening has made it a powerful detection tool in bacterial genomics [50]. A Cas9nAR-assisted lateral flow strip was used to identify two food-borne pathogens without the need for specialist analytical equipment, allowing it for the simultaneous amplification of two pathogens of interest in a mixed sample and immediate visualization of the detection in 3 h causing

enhanced selectively and sensitively with a LOD (100 CFU/mL) [51].

Salmonella is the most common causes of foodborne infections posing a substantial health risk in United States with over 2600 different serovars, causing diarrhoea, fever, and stomach cramps post-infection. According to the WHO, about one million cases of reported salmonellosis are resulting in 20,000 hospitalizations and 400 morbidities annually [52,53]. *Salmonella* spp. strains are zoonotic MDR pathogens causing several outbreaks as reported by CDC. It acquires resistance in the animal host before being transmitted to humans via the food chain [54]. A variety of foods products including sprouts, chicken meat products, eggs, cucumbers, dry pepper, tuna, and tomatoes, have become increasingly linked to *Salmonella* outbreaks over the last decade. It can infect the food supply chain at any point throughout the manufacturing, processing, distribution, or marketing process. Because of the high frequency of *Salmonella*, effective methods for timely identification, detection, and monitoring are required. Detection of small numbers of microbial cells in food and clinical samples are incredibly useful, but it is still a major challenge [55].

Due to the use of culture-based techniques, current surveillance for this pathogen is limited to the detection of only the most abundant serovars. Thus, pathogenic serovars present in a small number of people remain undetected [53]. The CRISPR-based analysis aids rapid genotyping for future perspectives of infection identification and pathogen detection. The evolution of *S. enterica* sub-lineages is primarily driven by either the loss of coding regions with established metabolic responsibilities for functional decline or the acquirement of horizontally transmitted phage and plasmid DNA causing activation of virulence and resistance [56]. The CRISPR in *S. enterica* acts as a defence tool against invading plasmid as well as phage DNA, by altering the phenotype for CRISPR-mediated temporary immunity, regulating long-term sub-lineage development [56]. CRISPR analysis of spacer content of *Salmonella* was

Fig. 2. Detection mechanisms of using CRISPR-Cas against different food-borne pathogens. Schematic illustrating ASFV detection in suspected swine serum samples using the CASLFA technique along with, the CRISPR/Cas9-mediated test strip based on the DNA HRP-AuNP probes. DNA-HRP-AuNP probes are pre-embedded in conjugate pads. Cas9/sgRNA recognizes the biotinylated DNA product, is added dropwise to the sample pad and then flows through the conjugate pad and hybridizes with DNA-HRP-AuNP probes. The resulting complexes flow through the test line and are captured. Excess DNA-HRP-AuNP probes flow through the control line and are captured by precoated single-stranded DNA probes. When the target DNA is detected, the test strip will show two colored bands that form through a reaction catalyzed by HRP with the peroxidase substrate DAB, and only one control line band will appear when no target DNA is detected (Figures adapted and edited from Refs. [136,159]).

significantly connected to serovar specific multi-locus sequence type, independently verifying evolutionary patterns and emphasising the value of CRISPR-based genotyping [57]. CRISPR1 and CRISPR2 are two CRISPR loci in *Salmonella* sp, and the spacer content of these can be regarded as serovar-specific. The evolution of CRISPR-Cas systems for conservation of Cas gene, direct repeat organization, leader sequencing and protospacer pairing can be used as a detection tool for *Salmonella* identification [58]. The utilization of serovar-specific well-conserved CRISPR-Cas spacer content has become a unique way for genotyping, leading to easy and rapid detection. CRISPR-Sero Seq is an amplicon-based multiplexed sequencing technique for detecting several serovars in a single sample that was able to confidently detect a serovar constituting of 0.01% of the sample using mixed gDNA from two *Salmonella* serovars [53]. A detection technique (dubbed "APC-Cas") has been developed utilizing a three-step amplification to produce robust fluorescence signals, identifying low quantities of a bacterial pathogen without isolation. APC-Cas utilizes a combination of allosteric probes based on nucleic acids and CRISPR-Cas13a with an aptamer domain for detection of *S. enteritidis* [59]. The efficiency of signal amplification of APC-Cas was successfully substantiated by recognizing low cross-reactivity of *S. enteritidis* with other pathogens. It is applied for the detection and quantification of *Salmonella enteritidis* cells ($1–10^5$ CFU) in a variety of samples, including milk, with sensitivity and accuracy comparable to or better than traditional real-time PCR [59].

The gram-positive bacterium *Listeria monocytogenes* is a common opportunistic food-borne MDR pathogen causing listeriosis in elderly, immune-compromised patients, foetuses, and infants, with a case of 30% fatality rate. The disease is spread mostly by the eating of contaminated food [60,61]. For precise detection of this infection rapid and sensitive technologies are necessary during food production. The detection of *Listeria* cells using CRISPR-Cas-assisted phage engineering via bioluminescence-reporter bacteriophages, through *de novo* genome assembly and activation using NLuc enzyme, has been incorporated into wide host range phage (A511) for enhanced sensitivity. The two serovar-specific *Listeria* phage responsible for reporter proteins are detected by NLuc-based phage within one day when present in samples such as milk, lettuce, cold cuts [62]. CRISPR-based subtyping has become feasible due to next-generation sequencing and new bioinformatics tools. CRISPR analyses saves time and is economical compared to WGS subtyping [63]. However, not all bacteria carry CRISPRs (approximately 48% of all bacteria), and hence are not considered as a good candidates for CRISPR-based subtyping [31].

Similarly, *Shigella* is a MDR food contaminated infectious pathogen, causing 600,000 morbidities worldwide annually in USA and China [64–66]. Even though single doses of norfloxacin and ciprofloxacin have been exhibited to be beneficial against shigellosis but are rendered ineffective owing to the emergence of AMR through point mutation, cellular impermeability, active efflux system, mutation(s) at target locations, and horizontal resistance gene transfer [67]. *Shigella* regulates the activity of the CRISPR-Cas system on its own, acquiring an external gene for survival. The insertion sequences cause mutations in bacterial genome by interfering expression of target sequences through transcription and translation alteration. Cas genes have the potential for detection of genetically transferred AMR strain, causing the alterations in the CRISPR-Cas machinery, based on the mechanism of CRISPR-Cas-mediated horizontal transfer of AMR genes in *Shigella* [68]. A quick and sensitive detection is required owing to the low infectious dosage of *Shigella* sp and thus introduction of Spa gene as a novel sequence for *Shigella* detection in any species of *Shigella* aids in its detection. An AuNPs-based optical geno-sensing system for the detection of four *Shigella* sp has been utilized wherein, AuNP-DNA probes were hybridized with the Spa gene sequence to the complementary target for inhibiting and detecting the *Shigella* sp from aggregating in an acidic environment with a LOD and LOQ (8.14 and 26.6 ng/mL) [69].

Overcoming the few limitations in the application of the food industry for the detection of food-borne pathogens, we are witnessing that the CRISPR-Cas tool has made a revolution in theragnosis with a variety of approaches for the most prevalent MDR food-borne pathogens including *S. aureus*, *E. coli*, *Salmonella*, *Listeria*, *Shigella*; making it the most reliable method over other easily available, less sensitive molecular and biochemical methods.

3. Anti-microbial activity using CRISPR-cas complex for food pathogens

CRISPR based antimicrobials are attractive substitute to antibiotics which possess their potentiality to target harmful and commensal pathogen indiscriminately. Which is due to their specificity and programmability. In beneficial bacteria, they are useful in minimizing the dissemination of antibiotic resistance or virulence factors over different strains and, controlling cell proliferation. In addition, they are beneficial for elimination of contaminants in the mixed food crops without any adverse effects on non-specific strains. Moreover, CRISPR-Cas are utilized to target harmful pathogens that causes spoilage in food materials [70]. CRISPR-Cas is often used as a pathogen clearing machinery of cells through the double-stranded cleavage of bacterial genomes, lacking the robust DNA repair pathways [71]. In spite of facing numerous challenges during modification genome, CRISPR-Cas enables antimicrobial to cause lethal damage to MDR food pathogens in a sequence-specific manner. CRISPR-Cas targets the bacterial genomes, showing the self-targeting lethal effect on host, which results in a non-reversible reduction for antimicrobial action as illustrated in Fig. 3.

Despite possessing difficulty in isolation of strains due to their similarity in physiological properties, mixed culturing offers a wide range of advantages and disadvantages [72]. However, traditional methods of isolation or control of a mixed population have only provided partial solutions, the native CRISPR-Cas systems function as an effective antimicrobial and capable of selectively eliminate targeted strains or control the mixed culture in a dose-dependent manner [73].

CRISPR-Cas system become a unique antimicrobial with native CRISPR by providing a cell spacer which is transmitted through a plasmid with a self-targeting spacer [73]. The two sub-strains of *E. coli* share 99% sequential homology and have identical cellular processes that could effectively be separated by using the CRISPR-Cas system [73]. Since, the spacer sequence for the strain of interest can be developed by focusing variable genomic characteristics, the specific nature of CRISPR antimicrobials is incomparable in terms of point of specificity. CRISPR-Cas is a potent antimicrobial system to control the food pathogens or spoilage organisms, such as *E. coli*, *Enterobacteriaceae*, and *S. aureus* through the splitting of the genomes of target organism. Thus can be proved as a potent tool for pathogen clearance [74–76]. Interestingly, CRISPR mediated antimicrobials have been tested for the determination of strains having an absence of native CRISPR-Cas systems [75,77]. The characteristics of the CRISPR antimicrobials have been confirmed by the analysis of the differentiation potential between two strains in a single base pair [75]. Antimicrobials are also manipulated to handle targeted unwanted antibiotic resistance or virulence genes for the removal of strains [75]. A genome-wide library of guide RNAs constituting specific five-nucleotide seed sequences was used to drive catalytic dCas9 to stop gene transcription to deficits severe fitness or even kill *E. coli* regardless of their fifteen nucleotides in the guide sequence. These consequences are intensified by high dCas9 concentrations and mitigated by fine-tuning dCas9 expression while retaining robust on-target suppression. The antimicrobial role of the CRISPR-Cas complex was illustrated for off-targets with nine nucleotides of similarity to the guide RNA that can significantly suppress gene expression [78]. The Cas proteins genes in combination with crRNAs, and CRISPR array promoters degrade and penetrate foreign DNA in *Escherichia coli*. A promoter incorporated CRISPR directs the transcription of precursor crRNA, whereas the other promoter directs transcription of Cas gene upstream of the protein-coding genes. H-NS, the DNA-binding protein is involved in expression of Cas by suppressing the CRISPR-Cas promoters, causing
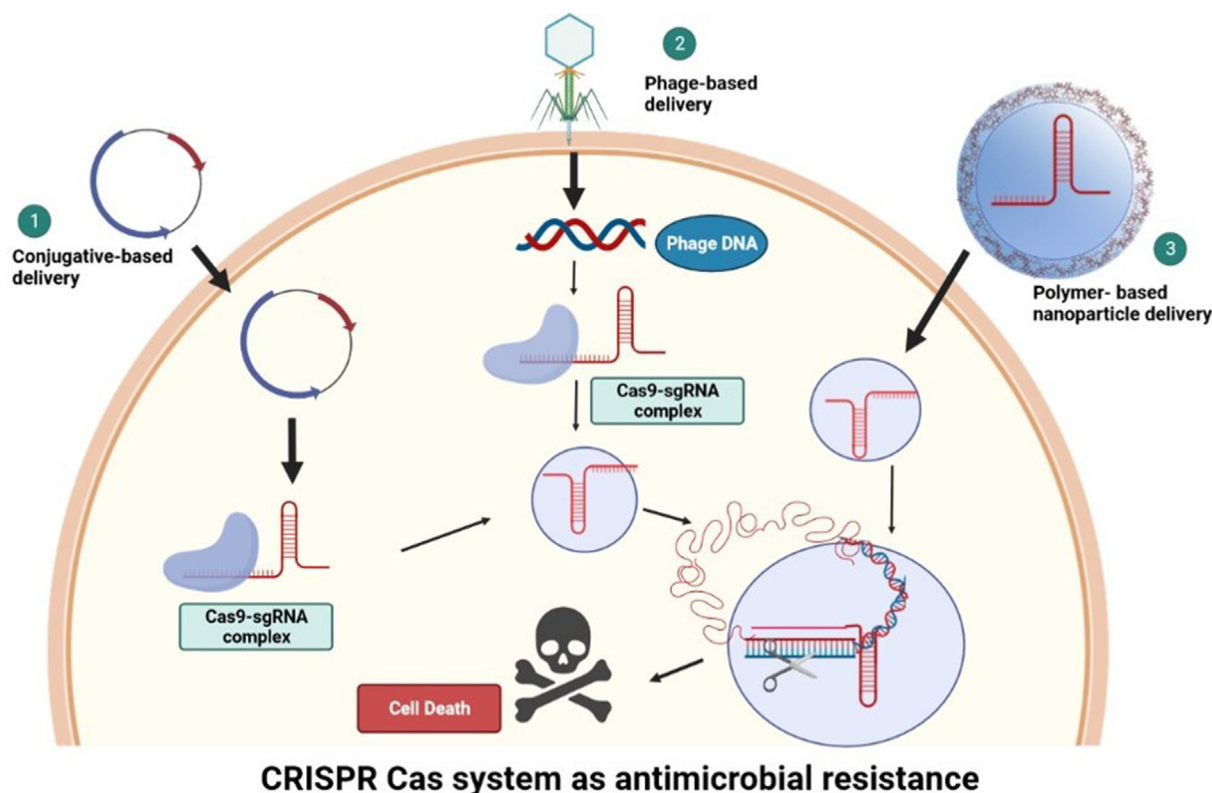
Fig. 3. Graphical model demonstrating CRISPR-based delivery through three different mechanisms targeting antimicrobial activity **1.** Conjugate-based delivery, **2.** Plasmid-based delivery and **3.** Polymer-based nanoparticle delivery.

anti-microbial activity using bacterial own defence systems [79]. Thus, CRISPR-Cas mediated antimicrobials deliver a rapid and comprehensive method for selection of certain strains without any effect on other closely connected strains. Additionally, they can be used for various purposes, like further research in identification of a strain, desirable elimination of contaminant from a media, to screen undesirable genes, and culture handling for optimal product quality. However, these application faces several obstacles. For example, the requirement of a suitable delivery system for the maintenance of populations of strain, particularly for those lacking native CRISPR-Cas systems. Small host range limits the usage of phage mediated delivery [73]. Targeting CRISPR is not always 100% effective because sequences in both the host and target mutate to escape CRISPR-Cas cleavage [71]. Further research focused to explore the causes of the CRISPR escape is required for the improvement of effectiveness of CRISPR antimicrobial.

4. Phage resistance of food pathogens using CRISPR-cas complex

Phage is primarily responsible for slow fermentation in the food industry because it causes contamination of starter cultures and are resistant to physiological treatments, such as, thermal processing, pasteurization, high pressure, and radiation technology [80]. Food industries have developed several ways, including changes in factory design and sanitation to minimize any economic losses associated with phage infection. The pathogens aiding in fermentation utilize CRISPR-Cas against invasive phage. The manifestation of CRISPR-Cas system is very much predictable in fermentation micro-organisms given the predominance of phage during fermentation processes. The adaptive and interacting nature of CRISPR-Cas indicates its capability for acquiring a spacer which encounters to be immune to this phage. The immunity-providing effect of native CRISPR-Cas systems is relatively simple and does not require the necessary engineering to promote the system to adapt to new spacers [81]. CRISPR-Cas system from

S. thermophilus was transplanted into *E. coli* against phage infection by heterological protection. The sequence-specific CRISPR-Cas based interference causes alterations in the proto-spacer adjacent motif, evading CRISPR-encoded immunity in a McrA/HNH- and RuvC/RNaseH-motifs dependent manner. The transmission of active CRISPR-Cas systems across distant genera is the reason of heterologous interference generation against invading nucleic acids [82]. The available published outcome from the researcher across the globe is comprehending that not only for thero-diagnostics but CRISPR-Cas is also contributing to limiting food contamination and fermentation failure by phages, which were previously supposed to be attenuated by resistant or mutant microbes, those which have the potentiality to cause food borne diseases. Hence CRISPR-Cas is playing a significant role in the dual application of preventing phage-induced contamination as well as inhibiting the application of resistant pathogens for preventing phage contamination.

5. Plasmid vaccination of food pathogens using CRISPR-cas complex

CRISPR-Cas systems depict the intake of plasmids being prevented by dividing the DNA [83,84]. When a cell possessing CRISPR-Cas based immunity containing plasmid encounters, it causes the protospacer to be vaccinated against the uptake of that plasmid, resulting in evolution of strain through CRISPR-mediated vaccination. Another advantage gained from plasmid vaccination is the hindrance of unwanted DNA elements responsible for pathogenicity islands or antibiotic resistance genes, which are transmitted via plasmids [85–87]. The spread of AMR is a particular concern for the food industry and the occurrence of CRISPR-Cas was associated with an absence of AMR markers in *Enterococci* sp [87]. Moreover, phage resistance is engineered for CRISPR-Cas targeting plasmid-contained antibiotics-resistant genes in strains containing native CRISPR-Cas systems. This was successfully performed in *S. thermophilus* by selection of a strain without plasmid presented by a

AMR gene containing plasmid [82].

6. Strain typing of food pathogens using CRISPR-cas complex

CRISPR-Cas is a potent technique not only for the detection of strains but also understanding the stress difference in microbial ecology investigation and development of environmental complex. Thus, providing epidemiological insights and understanding of populations of the genotypes [88,89]. Strain typing, a novel and reliable tool for sequencing CRISPR repeat-spacer arrays, has limitation of its application due to the occurrence of CRISPR-Cas in the strains of interest. However, industry related to starter culture is particularly favourable to the use of CRISPR-Cas in characterisation of strains because of the availability of CRISPR-Cas in many microorganisms related to fermentation industry [90].

CRISPR-Cas is favourable for strain-typing applications in microorganisms of fermentation process because of its high occurrence in food pathogens [90]. The utilization of repeat spacers for strain typing in *S. thermophilus* in the production of cheese and yogurts provides insight into the connectivity of different strains and their ecology [91]. The repeat-spacers of Cas genes were studied for the identification of *Lactobacillus casei* and *Lactobacillus paracasei* as probiotics [92]. *Lactobacillus rhamnosus* and *Enterococcus faecalis* based CRISPR genotyping could distinguish between related strains in handcraft and industrial dairy and meat products [93]. Sequence analysis of well-known commensal and probiotic microorganism *Bifidobacterium genus* and *Lactobacillus gasseri* exhibit genetic divergence while CRISPR-Cas based strain typing monitors the existence of spoilage microorganisms, in many fermentation processes [94,95]. Moreover, *Salmonella* sp, *Lactobacillus buchneri*, *Campylobacter jejuni, Clostridium difficile, Corynebacterium diphtheriae, E. coli, Vibrio parahaemolyticus* have also been genotyped via CRISPR-cas complex for stain typing and their application in food industry [96]. Thus, the CRISPR-Cas strain typing system is utilized to ensure the safety and quality of the food product, regardless of whether spoilage could be tracked or food pathogens are identified. Hence, with all reference to these progressive and prosperous applications, further research outcomes are expected with the data diminishing the limitation of the application of the CRISPR-Cas system in strain typing while enhancing its usage for preventing pathogenic outbreaks by available sequence identification.

7. Genome editing of food pathogens using CRISPR-cas complex

CRISPR-Cas are used as a tool for genome editing by Type II systems owing to their complex interference, simplicity in application, and the sequence-specific way of targeting DNA as illustrated in Fig. 4 [97]. For genome editing through CRISPR-Cas, four elements need to be established which includes the Cas9 protein effectors and the crRNA-tracrRNA complex. The crRNA-tracrRNA complex interacts with the target sequence of DNA and DNA repair mechanism within the host organism to repair the damage done by CRISPR-Cas for incorporation of the desired genomic changes [98]. Even though the pathogens contain a native CRISPR-Cas-type II, it probably contains no spacer that can focus precisely to the editing point. sgRNA has revolutionized the genome-editing field since sgRNA can be easily be developed for effectively targeting of genome of interest for Cas-9 delivery system. sgRNA simplified the two-component system of Cas9 and sgRNA which was easy to plan, package and transfer the cells of interest [97]. The vast bulk of genome editing of CRISPR in eukaryotes occurs when the cell performs its damage repairing machinery through endogenous DNA repairing mechanism like homology-orientated repairing, resulting into mutations on the site of cleavage [99]. However, CRISPR-Cas are used to modify the genome due to the lack of robust DNA repairing mechanisms in the bacterium, hence physiology and homeostasis mechanisms such as cleavage of double-strands of bacterial genomes causes cell death [98].

S. thermophilus in fermentation industry and *Lactobacillus reuteri* in probiotics exploits CRISPR-Cas to select certain genotypes [71,100].

CRISPR-Cas was utilized in *S. thermophilus* for selection of heterogeneous populations based on naturally occurring genotypes [71]. Using self-targeting of CRISPR-Cas, the individual genotypes for genomic islands of interest was screened. A combinatorial platform was developed with CRISPR-Cas to use ssDNA recombinant engineering, and enhance recoverable recombinants in *L. reuteri* to select mutants that were recombined with ssDNA [100]. CRISPR-Cas9 endonuclease complexed with dual-RNAs are used for the introduction of mutations in the genome of pathogens, eliminating the requirement for selectable markers and counter-selection systems through dual-RNA-Cas9 directed cleavage at the targeted genomic nucleic acids for unmutated pathogenic clearance [101]. The sequence of crRNA was modified to perform single-nucleotide and multi-nucleotide editing to reprogram dual-RNA-Cas9 specificity. Multiplex mutagenesis occurs when two crRNAs are used simultaneously and transformable cells are selected and a minor manipulation at the target sequences by dual-RNA- Cas9 cleavage that aids gene editing in *S. pneumoniae* and *E. coli* for the selection against non-recombinant cells [101].

The combination of ssDNA recombination and CRISPR-Cas possesses the ability to recover low efficiency and target codon mutagenesis. Even though ssDNA engineering is an impactful mechanism, its application is challenging, as it must be optimized for each strain. The primary focus is on the application of CRISPR-Cas for homologous recombination. Nevertheless, the utilization of CRISPR-Cas in genome editing increases its application in the food industry against MDR food pathogens. Furthermore, characterization to implement minor and significant mutations of bacterial genomes is required. Moreover, CRISPR-Cas is used for the natural selection of genotypes by exploiting their functionality from industry insights.

8. Nanomaterials(NM) as an antimicrobial and detection tool against food pathogens

The applications of nanotechnology have gained quite an interest in the food industry due to their high physical, and chemical stability, magnetic properties, and large constant lattice values [102,103]. These features are mainly used in the food industry to enhance the overall quality, shelf life, taste, flavor, processing capacity of the food, and increased specific surface area for antibacterial activity [104] (Fig. 5). These applications can primarily be summarized into two main groups which include the nano-structured food matrixes and nano-sensor for food. Nano-structured food constituents covering the broad range of processing and packaging industries are also applied as food preservatives, smart carriers, anti-caking age and antimicrobial agents, and fillers to strengthen the mechanical properties of packing material. Furthermore, nano-sensors can be used to improve food quality and safety assessment [18]. Food-borne infections have emerged as a major public health concern and thus causing the need for both quick and sensitive testing of various food-borne pathogens. Several metal-based NPs (MNPs) and organic NPs (ONPs) have been used directly or upon functionalization for enhanced binding affinity with the pathogen for improving the surface selectivity, leading to pathogen detection with accuracy [105]. Herein, we have highlighted the clinical significance of NPs as an antimicrobial and detection tool against food pathogens as illustrated in Figs. 6 and 7.

The anti-bacterial properties exhibited by ZnO-NPs through cytoplasmic leakage caused by rupture of the cell membrane via abrasiveness and surface oxygen species for biocidal effects against *E. coli* DH5 and *S. aureus* owing to their stability under pressure through their interaction with pathogen [106,107]. The values of MIC and MBC suggest that nano-colloidal AgNPs possesses bacteriostatic effect but a weak bactericidal impact, against food-borne pathogens (*Salmonella enterica, Serovar Typhi, Escherichia coli, Listeria monocytogenes, Bacillus cereus, Vibrio cholerae, Vibrio parahaemolyticus, and Staphylococcus aureus)* and since they are inexpensive and exhibits great antibacterial efficacy, they could act as potential antimicrobial agents [108]. Alike chitosan, AgNPs impair
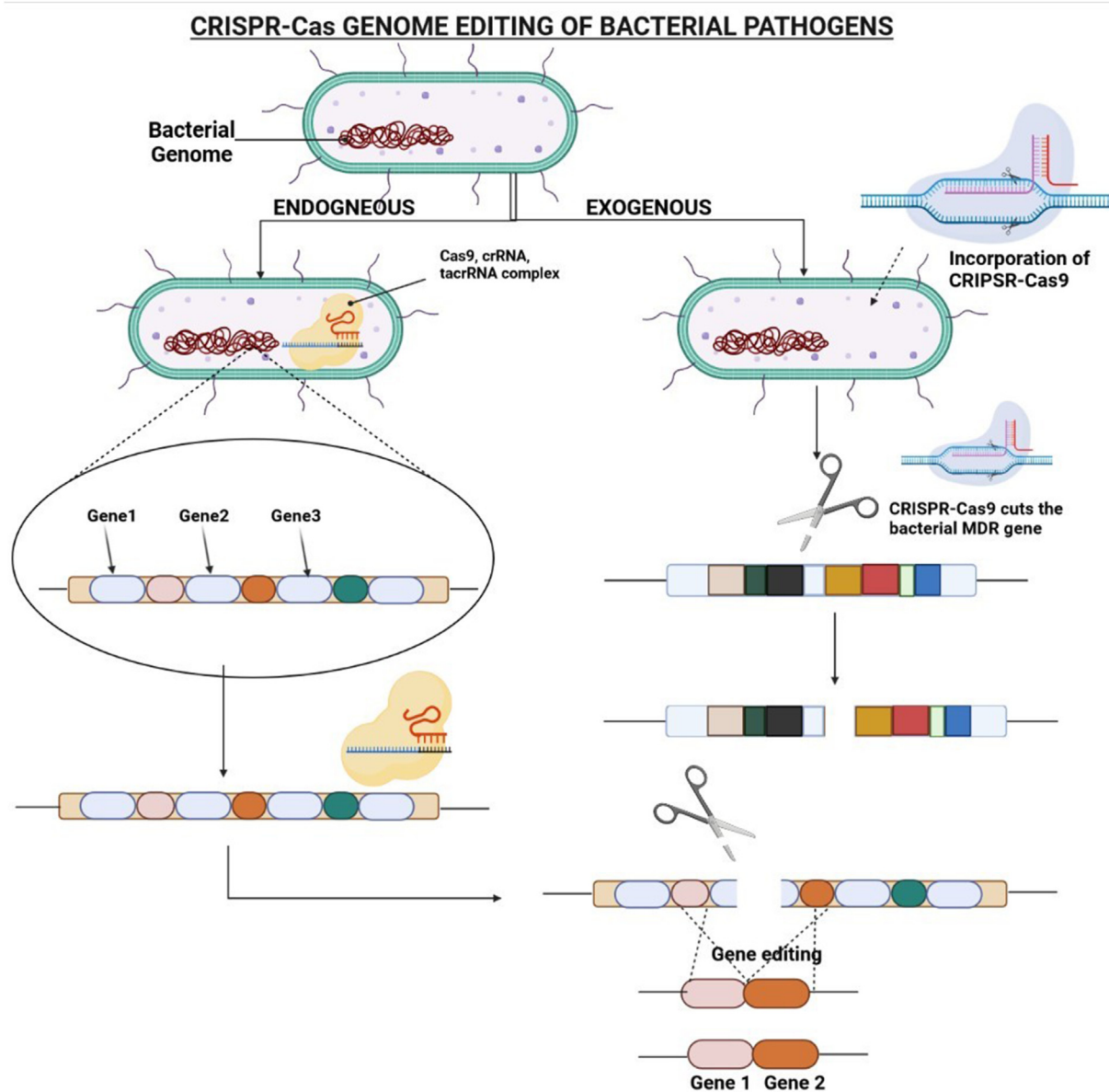
CRISPR-Cas GENOME EDITING OF BACTERIAL PATHOGENS

Fig. 4. Genome editing for inactivation of MDR pathogens via CRISPR-Cas system. (1) **Endogenous mechanism** - pathogens containing a native CRISPR-Cas-type II without any spacer focuses on genome editing at desired location. crRNA-tracrRNA present inside bacterial cells forms a complex when come in contact with Cas9 protein. This complex further goes and bind to the bacterial gene at the site where DNA repairing is must and interrupt this repair mechanism, resulting into mutation at cleavage site. (2) **Exogenous mechanism** - Pathogens which do not have native CRISPR are incorporated with CRISPR (with the help of nano-carriers), where it acts as a scissors to cut the MDR gene, resulting into antibacterial sensitivity.

bacterial cells causing the loss of the ability to permeate the membrane and impart anomalies in the regulation of membrane transport and denature nucleic acid [109]. The antibacterial activity of polyamines attached to the exterior surface of mesoporous silica particles for enhanced anti-bacterial efficacy. The enhancement is due to the covalent functionalization with *N*-(3-trimethoxysilylpropyl) diethylenetriamine against *L. monocytogenes* through interactions between the positive amine corona on the surface of NPs and the negatively charged bacteria membrane leading to the cell membrane disruption. NPs surface conjugated with polyamine is 100 times more potent than free polyamines for *L. monocytogenes* clearance [110]. A novel nano-carrier was self-assembled by coupling the magnetic properties of surface active maghemite NPs (core) with tannic acid (shell) in water to synthesize SAMN@TA hybrid against *Listeria monocytogenes*. The material is employed in the food sector as an affordable, efficient as well as eco-friendly anti-microbial nanomaterial [111]. Gold nanoparticles

(AuNPs) of size range 7–40 nm and AgNPs of size range 10–40 nm were demonstrated to be highly potent against Gram-positive as well as Gram-negative bacteria [112] such as *B. subtilis* (AuNPs), *P. aeruginosa* (AgNPs*), S. mutans* (AgNPs), *S. aureus* (both AuNPs and AgNPs), *E. coli* and *K. pneumoniae* (both AuNPs and AgNPs) [113–115]. AuNPs cause reduced membrane potential by disrupting ETC and a decrease in ATP synthesis for irregular cell shape, membrane degradation, and the loss of its protective function [116,117]. The morphological characteristics of Au-NPs and Pt-NPs were assembled with *Salmonella Enteritidis* and *Listeria monocytogenes* as bacterial NPs vehicles for utilizing bacteria as a vehicle to transport a nano-conjugated drug. Au-NPs and Pt-NPs are encapsulated inside the bacteria via a self-organization method without the disruption of bacterial cell walls [118]. Due to the morphological interaction between Au-NPs and the pathogens (*Salmonella enteritidis* and *Listeria monocytogenes*), the aggregation of Au-NPs between flagella or biofilm networks did not permeate the bacterial cell. Pt-NPs entered
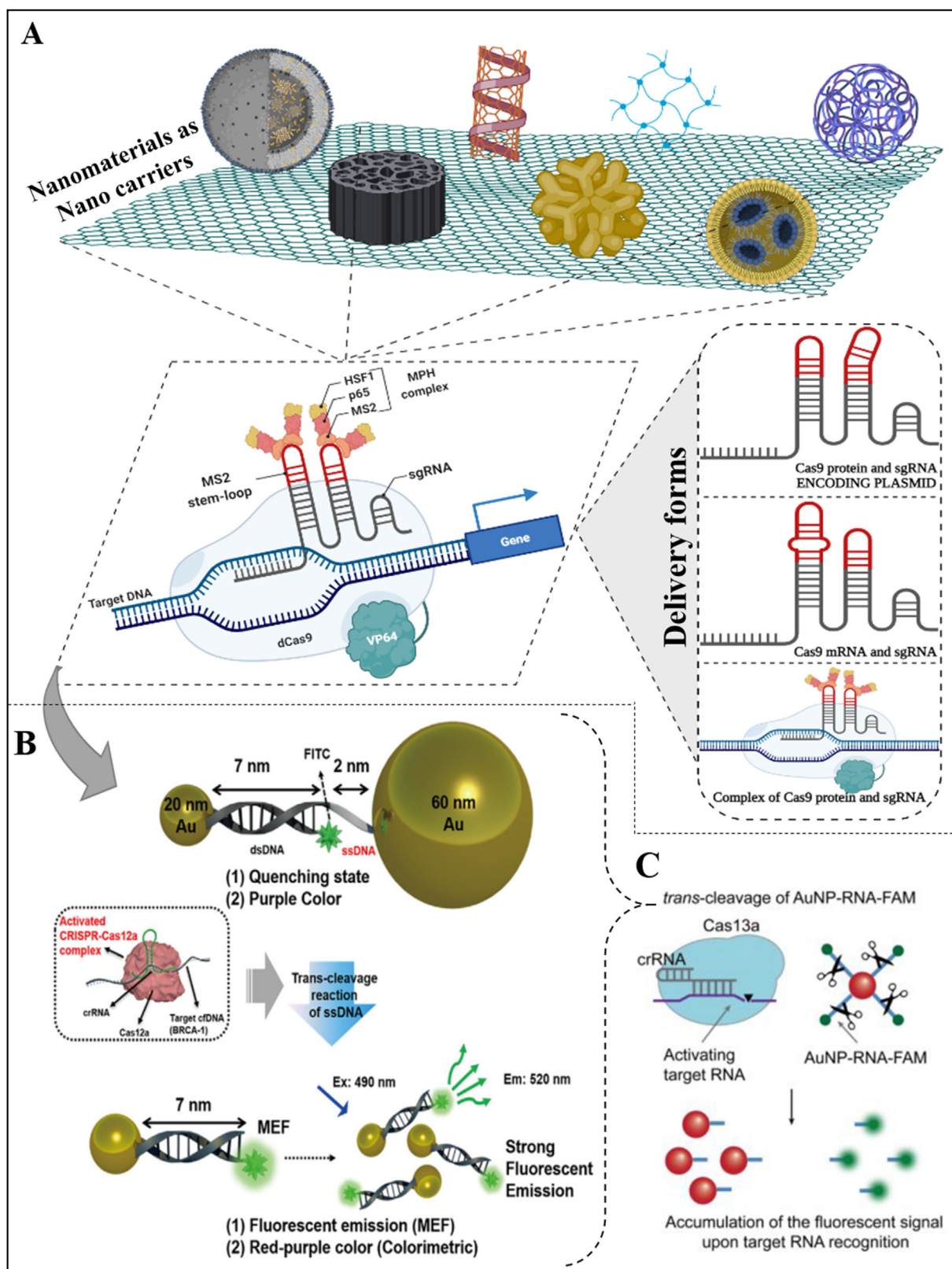
Fig. 5. (A) Different types of nanoparticles as nano carriers used for detection and antimicrobial activity of food-borne pathogens. **(B)** DNA-functionalized gold nanoparticles combined with the *trans*-cleavage activity of the CRISPRCas12a would release the short-distance gold nanoparticles, greatly increasing the fluorescence intensity. **(C)** Schematic illstrating the CRISPR/Cas13a detection system based on the AuNP-RNA-FAM probes (Figures adapted and edited from Refs. [159,160]).
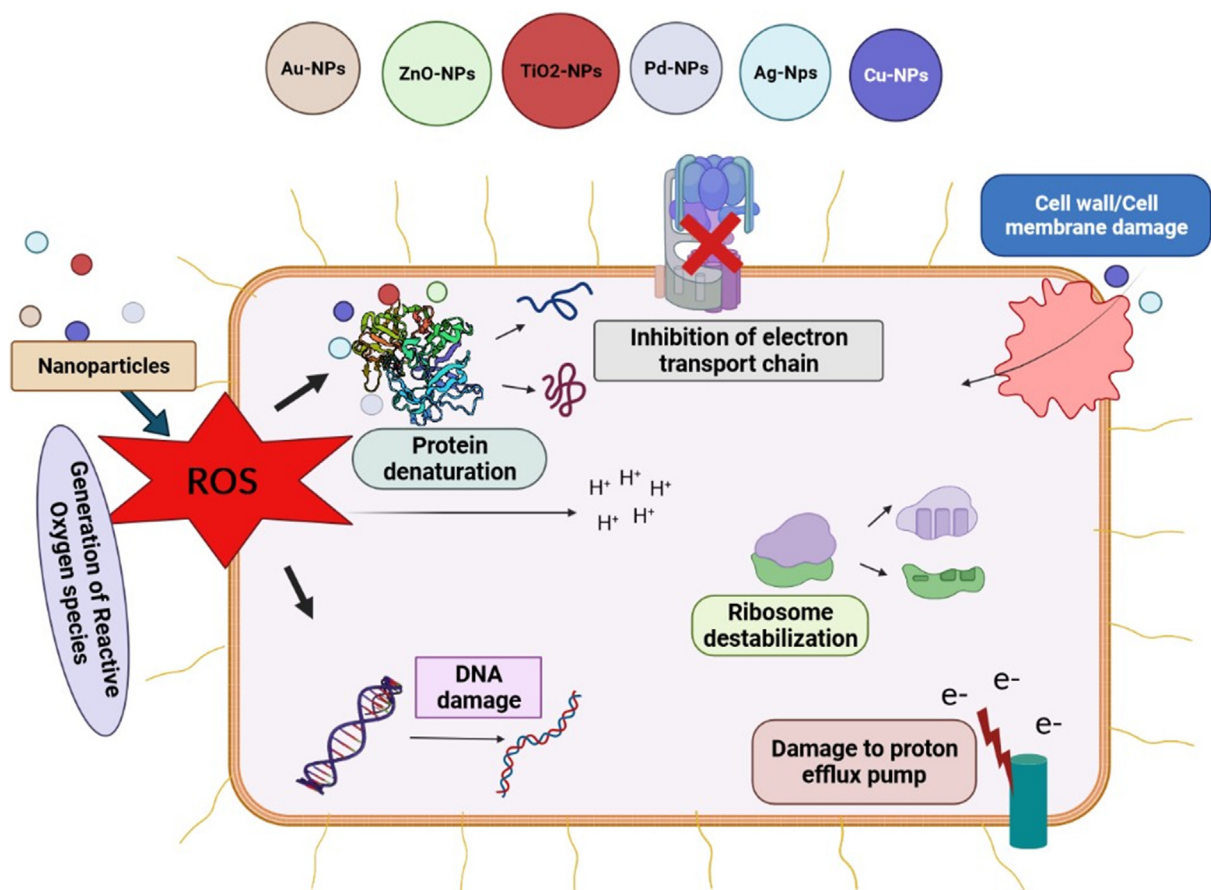
Fig. 6. Mechanism of Antibacterial action of different nanoparticles. The naoparticles act agai.

Listeria monocytogenes cells to cause elimination via its bactericidal properties from the cells [119]. Anisotropic dumbbell-like Au–Pt NPs pose efficient catalytic and anti-bacterial activity using a DNA-programming approach for the detection and eradication of bacterial infections [120]. Furthermore, the oxygen reduction activity of octahedral Pt–Ni NPs is attributed to shape selectivity and tailored surface composition [121]. Rhamnolipids functionalized liposomes (*rhamnosomes*) were produced using nisin for the induction of innate antibacterial and antibiofilm properties to enhance its efficacy against the foodborne pathogens. RSNVs depicted synergy between nisin and rhamnolipids for improved antibiofilm activity [122]. *Listerium monocytogees* were investigated in intestinal epithelium cells upon pre-treated with a low or high dosage of TiO$_2$ NPs for both adhesion and invasion. The increased invasion was exhibited due to cytoskeletal changes caused by NPs for upregulated levels of listeriolysin-O that protects *L. monocytogenes* from ROS for enhanced bacterial survival causing nano-toxicity. The potential risk of increased *L. monocytogenes* susceptibility related to the nanosized TiO$_2$ intake at small dose should be considered [123].

The identification of *E. coli* in the samples of ground beef using MNCs functionalized with biotin-labelled polyclonal goat anti–*E. coli* antibodies immobilized over streptavidin-coated magnetic NPs for MNCs. MNCs presented a MCE of 94% for *E. coli* and the immunoreaction time was 15 min deprived of any enrichment [124]. Carbon-based nanotubes both single-walled (SWNT) and multi-walled (MWNT) possess unique properties as an advantage with enhanced aspect ratio, high surface area, and unique electrical and optical aspects. Chemical modification or functionalization of carbon nanotubes employed for *in vitro* and *in vivo* evaluations ensures homogeneous dispersion in aqueous settings for pathogen detection [125,126]. A carbon nanotube functions as a 1-D

scaffold while being covalently connected with ᴅ-galactose molecules through ligand-receptor interaction by binding selectively with pathogenic *Escherichia coli* for cell agglutination [127]. For efficient detection of *E. coli* a micro-nanofluidic device functionalized with magnetic beads was utilized to capture the food pathogens through computational fluid dynamics (CFD), 3D-tomography, and machine learning to probe and bead stack the pathogen. Moreover, this nano-device is multiplexed, downsized, cost-effective, transparent, and easy to fabricate and operate which is making it ideal for pathogen separation in both laboratory and point-of-care (POC) settings [128].

The immunomagnetic MWNT is used as an alternative IMS platform for rapid and sensitive pathogen identification, owing to its ability to carry a large number of immuno-functionalities per unit weight of material while remaining stable in optically transparent liquids [126]. The use of IMS and MDA, preferentially concentrates *Salmonella* gDNA for improved detection of *Salmonella*-spiked raw chicken breast sample culture enrichment, allowing for serotyping and high accuracy SNP of food pathogens using shotgun sequencing has been implicated as a method of identification [129]. A specific, sensitive and accurate lateral flow immunoassay developed for the detection of *S. typhi* through QDNS, acting as signal reporters with visual LOD (5×10^3 CFU mL-1) for *Salmonella typhimurium* within 10 min [130]. Furthermore, non-*Salmonella typhimurium* bacteria were found in larger numbers depicts influence on *Salmonella typhimurium* detection [131]. *m*-AuNPs were characterized to selectively bind the mannose-specific adhesin FimH of type 1 pili by functionalizing with carbohydrate attachment as a labelling probe and multi-ligand carrier in biological systems. Thus, the robust specific binding of *m*-AuNP with bacterial type 1 pili paves a novel way for identifying particular proteins over the surface of the cell by using carbohydrate attached NPs [130]. A disposable immuno-sensor combining a
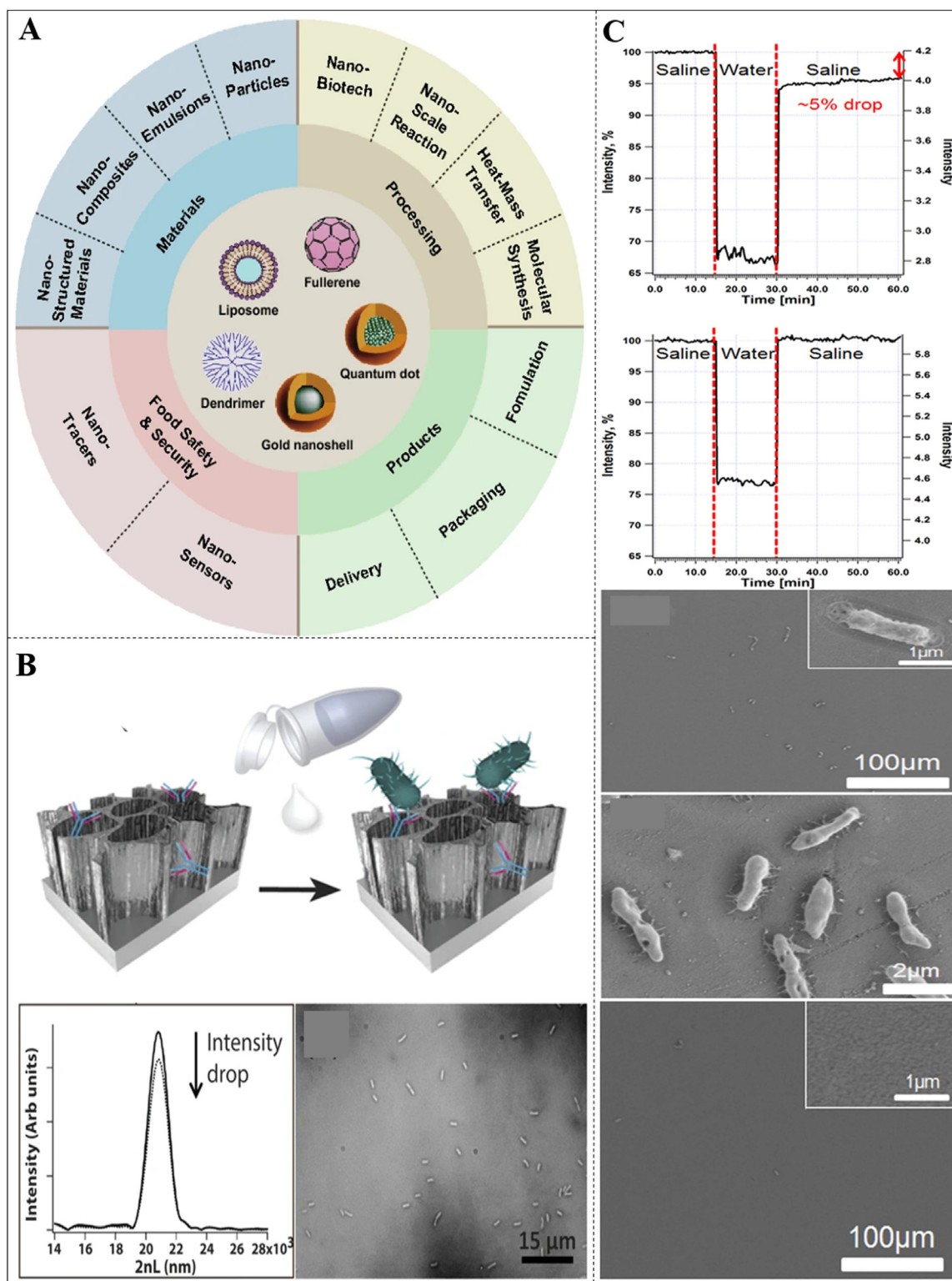
Fig. 7. A. Nanotechnologies in Food Science: Applications, Recent Trends, and Future Perspectives. **B.** Specific capture probes (antibodies) were immobilized onto the porous surface to provide the active component of the biosensor. The biosensor was then exposed to the target bacteria to directly capture the bacteria cells onto the antibody-modified PSiO$_2$ surface. A drop in the intensity of the thin-film optical interference spectrum of the biosensor results from bacteria capture. Microscopy tools (light microscope and HRSEM) and real-time PCR methods were used to confirm the presence of bacteria on the biosensor surface. **C.** Representative biosensing experiments and the corresponding HRSEM images of the biosensors immediately after the experiments. Spiked process water (104 cell/mL *E. coli*). Control - original process water (no *E. coli*). The corresponding HRSEM images (in two different magnifications) of the biosensor after a biosensing experiment with spiked process water, demonstrating bacteria capture. The inset presents enlargement of a captured bacterium on the biosensor surface. A corresponding HRSEM image of the biosensor after the control experiment (original process water, no *E. coli*) showing a negligible amount of cells. The inset presents enlargement of the biosensor surface (Figures adapted and edited from Refs. [161,162]).

magneto-immunoassay and AuNPs for electrochemical detection of *Salmonella enterica* by functionalizing a screen-printed carbon electrode (SPCE) with a permanent magnet. An anti-*Salmonella* magnetic beads (MBs-pSAb) were used to trap *Salmonella*-containing samples (skimmed milk), sandwiched with AuNPs-modified antibodies (sSAb-AuNPs) for detection using differential pulse voltammetry (DPV) causing 83% and 94% efficiency [128]. A label-free impedimetric aptamer-based bio-nano-sensor has been utilized based on electrochemically and chemical grafting of a diazonium-supporting layer onto SPEs which initiates immobilization of chemicals of aminated-aptamer, for capturing and detection of *Salmonella*. Furthermore, the cell systematic evolution of ligands by exponential enrichment was used to select DNA sandwich aptamers with excellent affinity and specificity for *S. enteritidis* detection. Sandwich capillary detection platforms utilized both aptamers crn-1 and crn-2 for recognition of target bacteria (*S. enteritidis*) precisely based on colour change with a detection limit (10^3 CFU/mL). Thus, this system is used to detect samples in food with a complicated matrix in a point-of-care naked eye setting [132]. A detecting system was established based on immune-magnetic NPs that incorporated magnetic and thermal properties of MNPs to allow efficient capturing, high sensitivity thermal sensor detection, and sterilization of *Salmonella typhimurium*. The spike in *Salmonella typhimurium* in drinking water can be detected with a detection limit of as low as 300 CFU/mL under ideal conditions [133].

A geno-sensor probe functionalized with a glassy carbon electrode was utilized for identification of *Listeria monocytogenes* in milk samples by modification of Pt-NPs dispersed in a chitosan matrix [134]. The DNA-based interfacial interaction between target DNA and Pt-NPs is immobilized with ssDNA and albumin to minimize non-specific DNA binding and increase signal sensitivity. It is a cost-effective, simple, user-friendly, and sensitive electroanalytical identification of gene sequences post hybridization with gDNA in the sample [134].

A highly sensitive fluorescence nano-bio sensor has been developed for the detection of *Shigella* species using two DNA probes for surface functionalization of AuNPs and MNPs. The target DNA was injected with the MNP-DNA probe and the DNA-AuNP-fluorescence DNA probe accompanied with a magnet used to separate the DNA probe complex while exhibiting enhanced sensitivity and specificity [135].

9. NM-CRISPR: A novel combinatorial therapeutic solution against food pathogens

Conventional diagnosis with limited stability and sensitivity necessitates the use of CRISPR as well as nanomaterials in the food industry to improve accuracy. Nanomaterials, primarily metal nanoparticles, offer a high degree of stability, as well as satisfactory optical and electrocatalytic properties, making them ideal for use as reporter molecules. CRISPR/Cas machinery on metal nanoparticles is being used in biosensor research to design a nanosensor with the motif of targeting a variety of pathogens [136,137]. Nanotechnology offers the potential to enhance the effectiveness and safety of CRISPR/Cas distribution, which has been known as a major limitation of its application. The combination of nanotechnology along with CRISPR/Cas technology holds a promising potential for next-generation thero-diagnostics of numerous food pathogens.

A spacer sequence containing crRNAs and tracrRNAs is produced by following transcription of CRISPR region and sgRNA processing. This sgRNA in conjugation with a protein complex possesses nucleotide degradation capabilities, allowing the DNA spacer sequence to be recognized by it [138]. The combination of sgRNAs and the CRISPR-Cas system triggered huge applications of genome editing for microbial detection and pathogen clearance. Despite great promises, the industrial application of the CRISPR-Cas system is inadequate due to lack of proper delivery. To overcome this aforementioned restriction several systems for delivering CRISPR-Cas and sgRNA to cells including viral and non-viral vectors. Nano-vesicles based on peptides, polymers, lipids, and extracellular vesicles are among the non-viral delivery systems. These nano-vesicles are designed to enhance the delivery by endosome escape

and environmental factors such as light, pH, and environmental features [139]. Despite progress in CRISPR-Cas development, the widespread practical efficacy remains unchanged. The conventional adenovirus or retrovirus systems are highly transfection efficient, but their toxicological characteristics, together with immune-response initiation and viral genome introduction inside host chromosomes, restricted their use as a delivery system [140]. Non-viral delivery systems, are safer and not limited by genome editing components, but are relatively low in their delivery efficiency [141]. Cationic lipid NPs, and cationic liposomes are utilized for gene delivery as non-viral delivery systems, due to the electrostatic interaction of negatively charged nucleic acids and CRISPR-Cas9 RNP complexes with the positive charge of NPs. Although cationic lipid NPs offer promising systems to provide genome-editing components, strenuous exploitation of Cas-9 due to poor stabilization of cationic lipid NPs with plasmid DNA or mRNA is challenging. The lipid nano-shells encapsulating genome-editing cargo are used to overcome issues pertaining to stability caused by exposure of cationic lipoplexes to serum. Herein, we have elucidated the strategies that can be incorporated for delivery of CRISPR-Cas utilizing nanocarriers and their preclinical significance as illustrated in Fig. 8, Fig. 9 and Fig. 10.

Most NPs enter cells through the endocytic pathways that include early fusion, maturation, and lysosomal degradation for efficient transmission of Cas-9 into the core to escape endosomes at the early stage. Liposome-mediated membrane fusion, cationic peptide-conducted membrane destabilization, PEI-mediated osmotic pressure, and pH-responsive polymer-mediated swelling are some of the endosomal escape strategies used to cause the fast release of nucleic or protein to the cytoplasm. A dendrimer encompassing glucuronyl-β-CD complex with Cas9-sgRNA is used to administer inside the cell, to induce gene editing efficiency at the injection site than ordinary Cas9-sgRNA conjugate [142]. A cationic diethylenetriamine movement for endosomal escape aids delivery of Cas9 mRNA and sgRNA by electrostatic interactions to the side chain of amphiphilic poly-aspartame derivates [143].

Phototherapy (PDT) represents another strategy for the induction or release of enclosed cargoes by laser-irradiated stimulation to deliver Cas9/sgRNA complex at the desired location and time. The light release via PDT aids in genomic modification at the target tissue while endosomal escape is facilitated, causing carriers to deliver their cargo. Photothermal as well as photodynamic activation studies have been conducted for intracellular delivery of Cas9-sgRNA plasmids [144]. A photothermal delivery system for plasmid DNA encoding Cas9 and sgRNA specific for the PLK1 gene has been developed, wherein a polymer derivative featuring semiconducting properties was grafted with PEI via thioester bond linkers. The transmission of dexamethasone from hydrophobic compartment of the polyplex contribute to nuclear-pore dilation, promoting the invasion of the Cas9-sgRNA plasmid [144].

A Glutathione-reducible synthetic lipid NPs was developed for the delivery of Cas9-sgRNA RNPs complexes [145]. Cas9-sgRNA RNPs can also be delivered through GSH-responsive disulfide bond-bearing cationic copolymers. Cas9-sgRNA-RNP complexes were also covered by the GSH-degradable cross-linker designed to supply Cas9-sgRNA, which were then polymerized by the formation of a disulfide bond for the NPs to cause degradation of GSH. Endosome escape occurs regardless of the cargo arrangement (DNA, mRNA, plasmids) encoding Cas9-sgRNA for delivering CRISPR via cell membrane penetration. The delivery system may become degraded by lysosomal enzymes once the endo-lysosome enters unless the endo-lysosome escapes via GSH delivery strategy [145].

A variety of pH-responsive polymers have also been introduced to make the endo-lysosomal release genome-editing materials. A derivative of polylysine-g-polyethylene glycol allows delivery of Cas9/sgRNA RNPs targeting STAT3 [146]. A variety of pH-sensitive amino lipids with modified amine head groups were synthesized for Cas9 plasmid delivery.

The intracellular regulation of genome editing components is being actively regulated by physical strategies, including needle-based membrane pored formation which induces effective genome editing through incorporation of the sgRNAs-Cas9 complex inside the cell. The silicon-
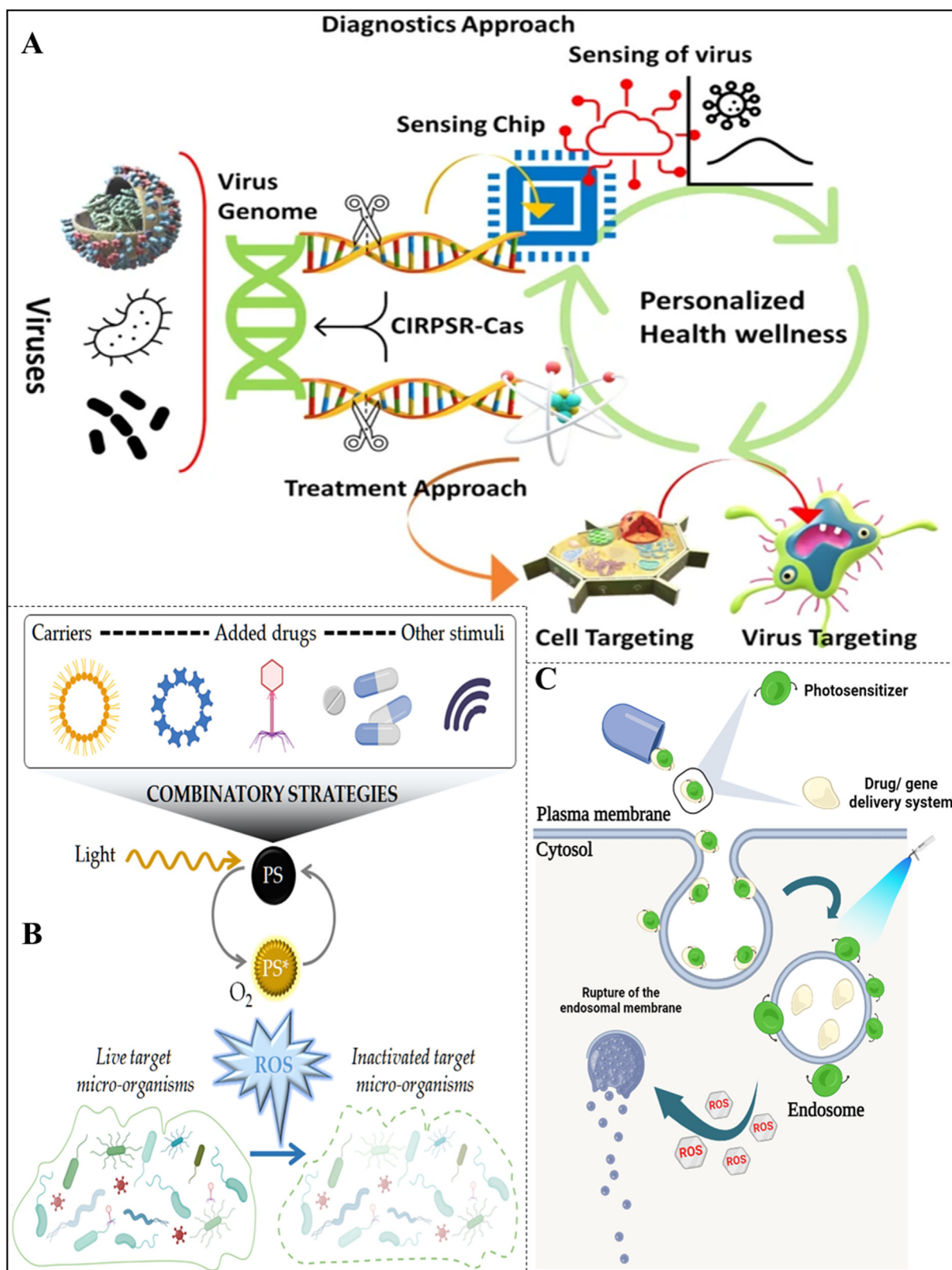
Fig. 8. Different strategies of CRISPR-Cas complex delivery by nanomaterials as a therapeutic solution against food-borne pathogens. **(A)** The perspective of nano-technology assisted CRISPR/Cas system for efficient diagnostics and treatment of a viral infection/diseases, overall, towards personalized health management. **(B)** Antimicrobial Photodynamic Therapy (pharmaceutics-13-01995-v3). **(C)** A pictorial representation illustrating the mechanism of photochemical internalization technology. The photosensitizer bind to the surface of the membrane. The gene/drug delivery system along with photosensitizer molecules internalize into the cells through endocytic pathway. The photosensitizers are integrated into the membranes of the endosomes and remain inactive. The photosensitizers becomess activated after light exposure and generates highly reactive singlet oxygen (1O2), causing rupture of the endosomal membranes. (Figures adapted and edited from Refs. [136,163]).
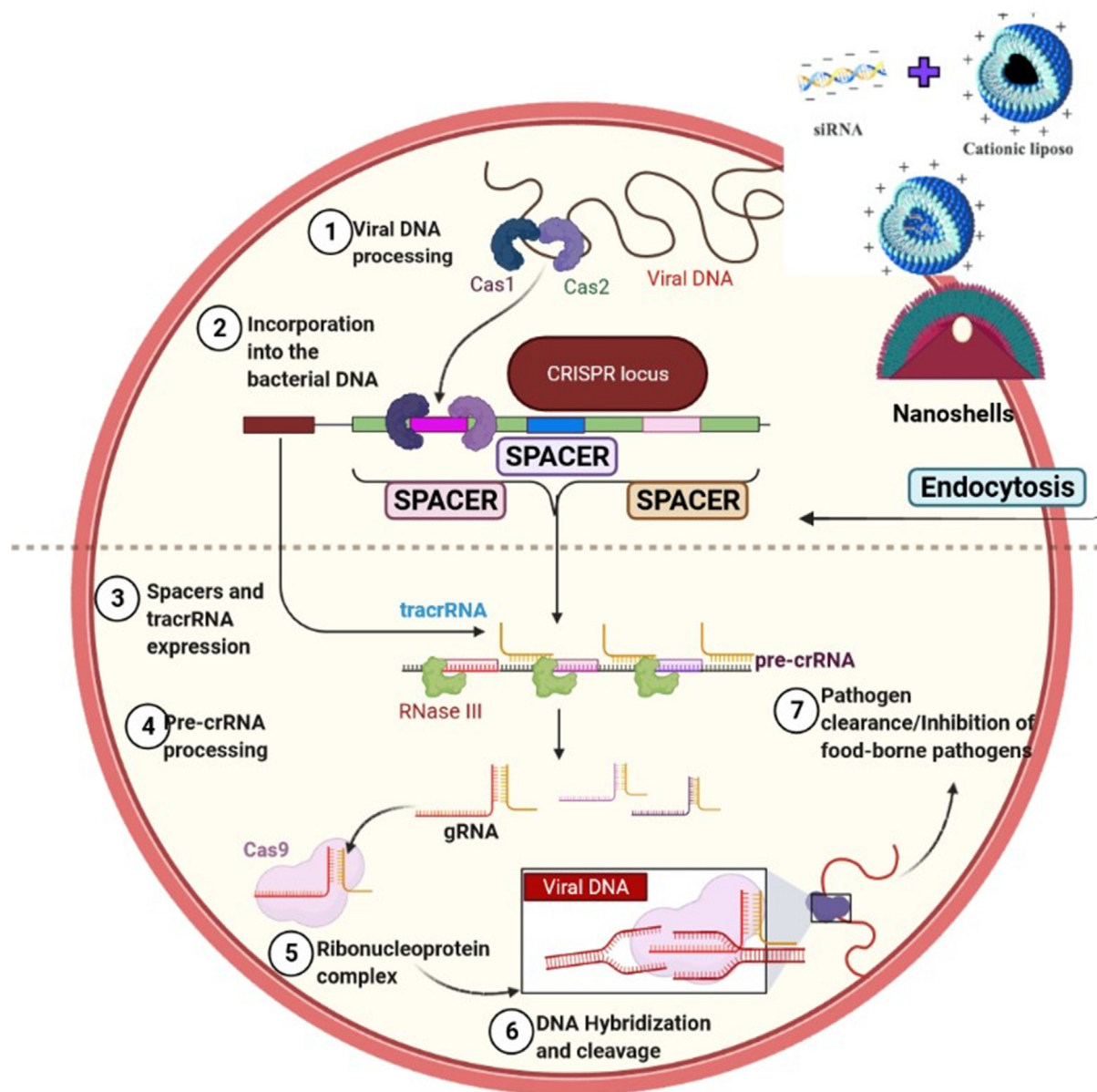
Fig. 9. Mechanism of Nano-CRISPR synergy for pathogen clearance.

based nanoneedle ranges with 200 nm diameter nanoneedles where the protein complex of sgRNA and Cas9 were adsorbed on nano-needles hydrophobic surfaces [147]. Nano-wires have also been used for delivering genome editing tools [148]. An efficient, rapid delivery is possible via physical approaches [149]. Various stimuli (mechanical forces, electrical forces) are used to deform the cell membrane for successful delivery. Physical strategies pose unique benefits of induction for effective genome control in cells that are difficult to transfect, such as hematopoietic stem cells while delivering cargo like the CRISPR-Cas system.

Kang et al. used a nanoscale CRISPR complex containing a polymer-derivatized Cas-9 endonuclease and sgRNA to target the mecA gene through covalent modification with branched polyethyleneimine for bacterial genome editing efficiently causing MRSA inhibition [150]. In another investigation gold nanoclusters (AuNCs) were utilized along with CRISPR -Cas9 to transfer the Cas9 endonuclease (SpCas9) from *Streptococcus pyogenes* into the nucleus via a highly pH-dependent assembly process. Thus, utilizing this novel strategy of combinatorial therapy will pave a new platform against the food industry.

Though multiple studies are illustrating this combinatorial approach of nanotechnology and CRISPR, several researchers are on their way to shrinking the toxicological impact of nanocarrier for CRISPR delivery, for utilization of this novel strategy of combinatorial therapy to pave a new platform for the food industry in combating food-borne MDR pathogens.

10. Challenges and outlook

Despite its high specificity and sensitivity, CRISPR/Cas-based combinatorial detection has some shortcomings, including the requirement for pre-amplification procedures along with a compatible host having a suitable delivery system with enhanced efficacy for reduction of off-targeting. One of the major challenges in the transformation of genome editing tools is the non-target effects of inefficient genetic systems on both CRISPR and cellular tools. The genetics of the off-targeted effect has been developed for new variants of CRISPR tools. Nevertheless, the specificity of the delivery system mainly depends on off-target side effects. The non-specific uptake of Cas9 NPs generates unexpected mutations in the immune cell causing lethal toxicity by deleterious off-target
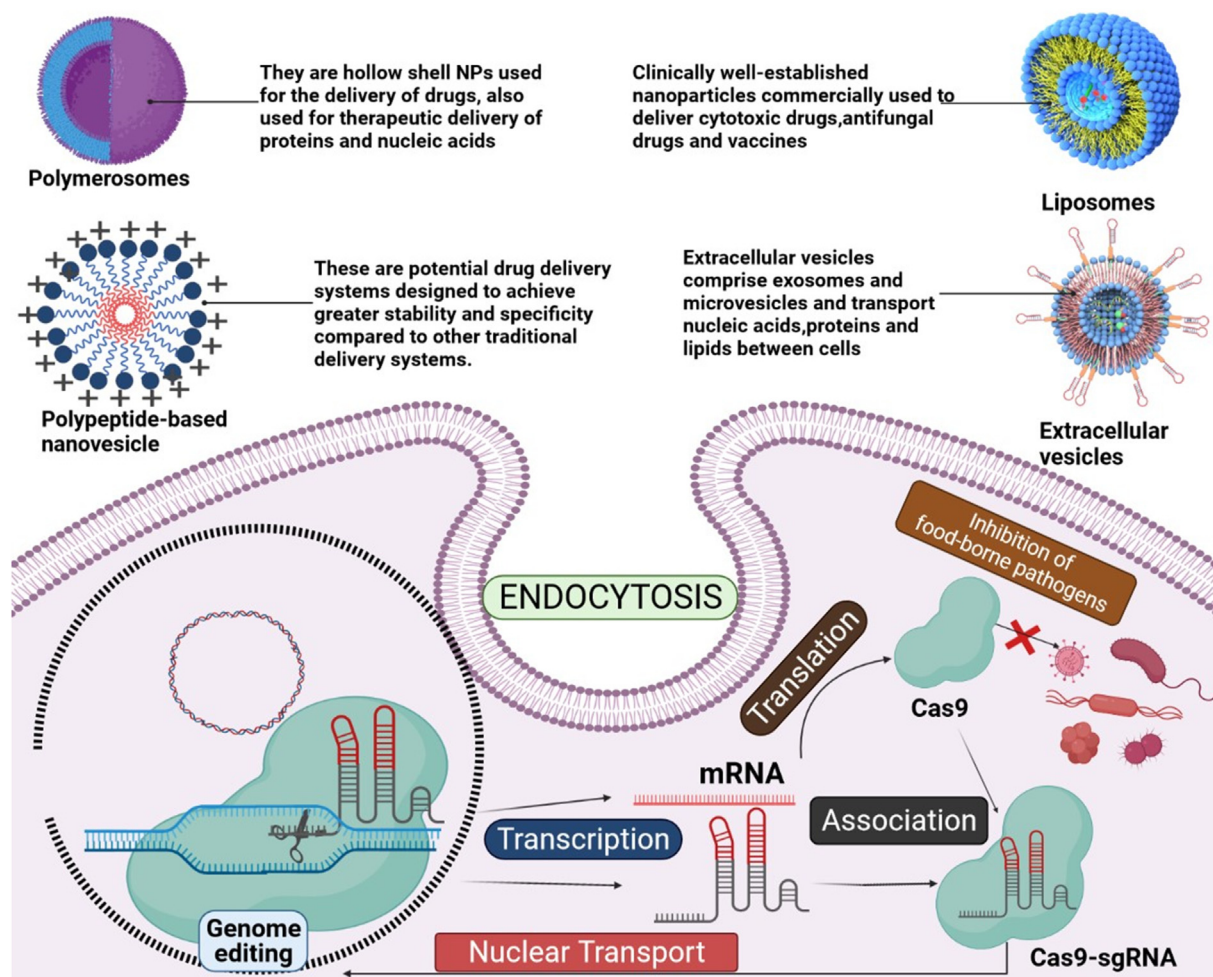
Fig. 10. Modified Organic nanomaterials for their use as CRISPR delivery and their mechanistic application with detailed molecular understanding of genome editing upon delivery.

mutations. So, future research focused to overcome these challenges, for preventing non-specific uptake by immune cells. Cas9/sgRNA delivery systems must be carefully considered for their route of administration in regular practice. Systemic administration allows particles in targeted tissue to be deposited deeply but causes unexpected gene-editing side effects due to their huge distribution volume in comparison with other routes of delivery.

Plasmid DNA expressing Cas9 and sgRNA have been exploited in some studies, but for these studies conducted successfully to the cytoplasm needs to contain the expression of Cas9 and sgRNA, and their complex need to invade into the nucleus for genetic modifications. The advantage of this single plasmid DNA-dispenser is easy, but its complex kinetics, complexity with sgRNA, and nuclear location reduce genome editing efficiency. The emerging platform for Cas9-sgRNA is natural vectors derived from extracellular vesicles. Although the biocompatibility and homeotropic-based targeting in these natural vesicles are superior, they pose several problems that should be addressed prior to their utilization for clinical practice. Based on the finding of the genome-editing machinery the detection of various pathogenic organisms has opened new doors to develop an effective, efficient and safe delivery system for pathogen detection.

Although several vectors for delivering CRISPR machinery have been studied in varied models but the field remains in its infancy. Additional modification shall be required for improving the limited efficiency of gene-editing, non-specific delivery, and unknown toxicity. Studies supporting regulatory perspectives are required for the approval of genome-editing components for future detection methods. Various strategies have

been exploited for promoting editing efficiency and minimizing off-target genome modification. Upon addressing long-term safety problems and clinical problems with CRISPR delivery, these powerful tools could be used successfully for clinical practise. Reduction of off-target modification, consideration of the antimicrobial administration rout, and the search of a suitable vector following the development of nano-sensors and improvement of nanotechnological application by diminishing the toxicological effects should be focus of future research to overcome these challenges for making a promising revolutionary combinatorial theragnostic tool of MDR food pathogens.

11. Conclusion

Food-borne pathogens are one of the major causes of infection worldwide, and the rapid increase in food-borne diseases has been the major area of concern for healthcare worldwide. In summary we have highlighted the application of the CRISPR-Cas complex as well as nanoparticles for rapid detection and antimicrobial properties against food borne pathogens. CRISPR based detection of food pathogen is gaining recent interest in the last couple of years wherein CRISPR/Cas9 and CRISPR-Cas 13a system, have been successfully employed for detection of drug resistant *S. aureus* and *E. coli* respectively (Fig. 7). The serovar-specific CRISPR-Cas spacer system has emerged as a unique approach for genotyping and fast detection of Salmonella. Moreover, CRISPR based sub-typing is the basis of detection of Shigella and Listeria too. Apart from the utilization as a detection tool, CRISPR has come a long way to be used effectively as a powerful antimicrobial against severe MDR food

pathogens by controlling the spoilage organisms and genetic editing tool. Not only as antimicrobial and detection tool but CRISPR-Cas system are also been used in case of fermentation industry for generating resistance to phages which are the main reason of slow fermentation. Another crucial application of CRISPR against food pathogens is plasmid vaccination, targeting phage resistance as well as antibiotic resistance. Strain typing and genome editing are some other efficient areas of applications of CRISPR-Cas system against severe food pathogens. Nano-compounds are also used in conjugation with some other assays (lateral flow immunoassay, immunomagnetic separation) as a successful detection method of food borne pathogens. Moreover, it is being utilized as an antimicrobial agent to inhibit the infection caused through contaminated food. Delivering the CRISPR system is the only limitation and utilization of nanocarrier nullified this problem. Herein we have presented with a novel combinatorial strategy of nano-CRISPR as a new potential therapeutic solution against food pathogens that elucidated the methodologies for the combinatorial therapy.

Data availability

All data included in this study are available upon request from the corresponding author.

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.

Acknowledgements

References

[1] E.S. Donkor, Cockroaches and food-borne pathogens, Environ. Health Insights 14 (2020), https://doi.org/10.1177/1178630220913365, 1178630220913365.

[2] S.P. Oliver, B.M. Jayarao, R.A. Almeida, Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications, Foodb. Pathog. Dis. 2 (2005) 115–129, https://doi.org/10.1089/fpd.2005.2.115.

[3] WHO, World Health Organization, WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015, Encycl. Parasitol. (2015) 1–265.

[4] J. Tanwar, S. Das, Z. Fatima, S. Hameed, Multidrug resistance: an emerging crisis, Interdiscip. Perspect. Infect. Dis. 2014 (2014), https://doi.org/10.1155/2014/541340.

[5] J.F. Lindahl, D. Grace, The consequences of human actions on risks for infectious diseases: a review, Infect. Ecol. Epidemiol. 5 (2015) 30048, https://doi.org/10.3402/iee.v5.30048.

[6] F. Pérez-Rodríguez, B. Mercanoglu Taban, A state-of-art review on multi-drug resistant pathogens in foods of animal origin: risk factors and mitigation strategies, Front. Microbiol. 10 (2019), https://doi.org/10.3389/fmicb.2019.02091.

[7] H. Nikaido, Multidrug resistance in bacteria, Annu. Rev. Biochem. 78 (2010) 119–146.

[8] B.M. Lund, Microbiological food safety for vulnerable people, Int. J. Environ. Res. Publ. Health 12 (2015) 10117–10132, https://doi.org/10.3390/ijerph120810117.

[9] R. Kumar, K.R. Aadil, K. Mondal, Y.K. Mishra, D. Oupicky, S. Ramakrishna, A. Kaushik, Neurodegenerative disorders management: state-of-art and prospects of nano-biotechnology, Crit. Rev. Biotechnol. (2021), https://doi.org/10.1080/07388551.2021.1993126.

[10] E. EFSA, European food safety authority and European centre for disease prevention and control, EFSA J. 13 (2015) 4329.

[11] S. Kunjachan, B. Rychlik, G. Storm, F. Kiessling, T. Lammers, Multidrug resistance: physiological principles and nanomedical solutions, Adv. Drug Deliv. Rev. 65 (2013) 1852–1865, https://doi.org/10.1016/j.addr.2013.09.018.

[12] F. Adzitey, N. Huda, G.R.R. Ali, Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks, 3 Biotech 3 (2013) 97–107, https://doi.org/10.1007/s13205-012-0074-4.

[13] F. Hille, E. Charpentier, CRISPR-cas: biology, mechanisms and relevance, Philos. Trans. R. Soc. B Biol. Sci. 371 (2016), https://doi.org/10.1098/rstb.2015.0496.

[14] Y. Li, S. Li, J. Wang, G. Liu, CRISPR/Cas systems towards next-generation biosensing, Trends Biotechnol. 37 (2019) 730–743, https://doi.org/10.1016/j.tibtech.2018.12.005.

[15] K.S. Makarova, E.V. Koonin, Annotation and classification of CRISPR-Cas systems, Methods Mol. Biol. 1311 (2015) 47–75, https://doi.org/10.1007/978-1-4939-2687-9_4.

[16] O. Tenaillon, J.E. Barrick, N. Ribeck, D.E. Deatherage, J.L. Blanchard, A. Dasgupta, G.C. Wu, S. Wielgoss, S. Cruveiller, C. Médigue, D. Schneider, R.E. Lenski, Tempo and mode of genome evolution in a 50,000-generation experiment, Nature 536 (2016) 165–170, https://doi.org/10.1038/nature18959.

[17] J. Zhou, L. Yin, Y. Dong, L. Peng, G. Liu, S. Man, L. Ma, CRISPR-Cas13a based bacterial detection platform: sensing pathogen Staphylococcus aureus in food samples, Anal. Chim. Acta 1127 (2020) 225–233, https://doi.org/10.1016/j.aca.2020.06.041.

[18] P.N. Ezhilarasi, P. Karthik, N. Chhanwal, C. Anandharamakrishnan, Nanoencapsulation techniques for food bioactive components: a review, Food Bioprocess Technol. 6 (2013) 628–647, https://doi.org/10.1007/s11947-012-0944-0.

[19] I. Zorraquín-Peña, C. Cueva, B. Bartolomé, M.V. Moreno-Arribas, Silver nanoparticles against foodborne bacteria. Effects at intestinal level and health limitations, Microorganisms 8 (2020), https://doi.org/10.3390/microorganisms8010132.

[20] M. Shafiq, S. Anjum, C. Hano, I. Anjum, B.H. Abbasi, An overview of the applications of nanomaterials and nanodevices in the food industry, Foods 9 (2020), https://doi.org/10.3390/foods9020148.

[21] A. Steinvil, Y.J. Zhang, S.Y. Lee, S. Pang, R. Waksman, S.L. Chen, H.M. Garcia-Garcia, Intravascular ultrasound-guided drug-eluting stent implantation: an updated meta-analysis of randomized control trials and observational studies, Int. J. Cardiol. 216 (2016) 133–139, https://doi.org/10.1016/j.ijcard.2016.04.154.

[22] S. Husain, S.K. Verma, Hemlata, M. Azam, M. Sardar, Q.M.R. Haq, T. Fatma, Antibacterial efficacy of facile cyanobacterial silver nanoparticles inferred by antioxidant mechanism, Mater. Sci. Eng. C 122 (2021), https://doi.org/10.1016/j.msec.2021.111888.

[23] P. Paul, S.K. Verma, P. Kumar Panda, S. Jaiswal, B.R. Sahu, M. Suar, Molecular insight to influential role of Hha–TomB toxin–antitoxin system for antibacterial activity of biogenic silver nanoparticles, Artif. Cells, Nanomed. Biotechnol. 46 (2018) S572–S584, https://doi.org/10.1080/21691401.2018.1503598.

[24] S. Kumari, P. Kumari, P.K. Panda, P. Patel, E. Jha, M.A. Mallick, M. Suar, S.K. Verma, Biocompatible biogenic silver nanoparticles interact with caspases on an atomic level to elicit apoptosis, Nanomedicine 15 (2020) 2119–2132, https://doi.org/10.2217/nnm-2020-0138.

[25] S.K. Verma, E. Jha, K.J. Kiran, S. Bhat, M. Suar, P.S. Mohanty, Synthesis and characterization of novel polymer-hybrid silver nanoparticles and its biomedical study, Mater. Today Proc. 3 (2016) 1949–1957, https://doi.org/10.1016/j.matpr.2016.04.096.

[26] M. Tertis, A. Cernat, S. Mirel, C. Cristea, Nanodevices for pharmaceutical and biomedical applications, Anal. Lett. 54 (2021) 98–123, https://doi.org/10.1080/00032719.2020.1728292.

[27] S.K. Verma, E. Jha, P.K. Panda, A. Thirumurugan, M. Suar, Biological Effects of Green-Synthesized Metal Nanoparticles: A Mechanistic View of Antibacterial Activity and Cytotoxicity, 2019, pp. 145–171, https://doi.org/10.1007/978-3-030-04477-0_6.

[28] S. Umesha, H.M. Manukumar, Advanced molecular diagnostic techniques for detection of food-borne pathogens: current applications and future challenges, Crit. Rev. Food Sci. Nutr. 58 (2018) 84–104, https://doi.org/10.1080/10408398.2015.1126701.

[29] I. Grissa, G. Vergnaud, C. Pourcel, The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats, BMC Bioinf. 8 (2007), https://doi.org/10.1186/1471-2105-8-172.

[30] B. Maji, S.A. Gangopadhyay, M. Lee, M. Shi, P. Wu, R. Heler, B. Mok, D. Lim, S.U. Siriwardena, B. Paul, V. Dančík, A. Vetere, M.F. Mesleh, L.A. Marraffini, D.R. Liu, P.A. Clemons, B.K. Wagner, A. Choudhary, A high-throughput platform to identify small-molecule inhibitors of CRISPR-cas9, Cell 177 (2019), https://doi.org/10.1016/j.cell.2019.04.009, 1067-1079.e19.

[31] I. Grissa, G. Vergnaud, C. Pourcel, CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats, Nucleic Acids Res. 35 (2007), https://doi.org/10.1093/nar/gkm360.

[32] D.G. Sashital, Pathogen detection in the CRISPR-Cas era, Genome Med. 10 (2018), https://doi.org/10.1186/s13073-018-0543-4.

[33] Lowy.D. Franklin, Staphylococcus aureus infection, N. Engl. J. Med. 339 (2001).

[34] M.W. Casewell, R.L.R. Hill, The carrier state: methicillin-resistant Staphylococcus aureus, J. Antimicrob. Chemother. 18 (1986) 1–12, https://doi.org/10.1093/jac/18.supplement_a.1.

[35] M.D. Sanford, A.F. Widmer, M.J. Bale, R.N. Jones, R.P. Wenzel, Efficient detection and long-term persistence of the carriage of methicillin-Resistant staphylococcus aureus, Clin. Infect. Dis. 19 (1994) 1123–1128, https://doi.org/10.1093/clinids/19.6.1123.

[36] S.Y.C. Tong, J.S. Davis, E. Eichenberger, T.L. Holland, V.G. Fowler, Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management, Clin. Microbiol. Rev. 28 (2015) 603–661, https://doi.org/10.1128/CMR.00134-14.

[37] J.L. Lister, A.R. Horswill, Staphylococcus aureus biofilms: recent developments in biofilm dispersal, Front. Cell. Infect. Microbiol. 4 (2014), https://doi.org/10.3389/fcimb.2014.00178.

[38] M. Kumar, Multidrug-resistant Staphylococcus aureus, India, 2013–2015, emerg. Infect. Dis. 22 (2016) 1666–1667, https://doi.org/10.3201/eid2209.160044.

[39] B. Goode, C. O'Reilly, J. Dunn, K. Fullerton, S. Smith, G. Ghneim, J. Keen, L. Durso, M. Davies, S. Montgomery, Outbreak of escherichia coli O157 H7 infections after petting zoo visits, North Carolina state fair, Arch. Pediatr. Adolesc. Med. 163 (2009) 42–48, https://doi.org/10.1001/archpediatrics.2008.525.

[40] J.P. Nataro, J.B. Kaper, Diarrheagenic Escherichia coli, clin, Microbiol. Rev. 11 (1998) 142–201, https://doi.org/10.1128/CMR.11.1.142.

[41] L. Bélanger, A. Garenaux, J. Harel, M. Boulianne, E. Nadeau, C.M. Dozois, Escherichia colifrom animal reservoirs as a potential source of human extraintestinal pathogenic E. coli, FEMS Immunol. Med. Microbiol. 62 (2011) 1–10, https://doi.org/10.1111/j.1574-695X.2011.00797.x.

[42] M. Aijuka, A.E. Santiago, J.A. Girón, J.P. Nataro, E.M. Buys, Enteroaggregative Escherichia coli is the predominant diarrheagenic E. coli pathotype among irrigation water and food sources in South Africa, Int. J. Food Microbiol. 278 (2018) 44–51, https://doi.org/10.1016/j.ijfoodmicro.2018.04.018.

[43] Z. Fu, S. Rogelj, T.L. Kieft, Rapid detection of Escherichia coli O157:H7 by immunomagnetic separation and real-time PCR, Int. J. Food Microbiol. 99 (2005) 47–57, https://doi.org/10.1016/j.ijfoodmicro.2004.07.013.

[44] X. Mao, L. Yang, X.L. Su, Y. Li, A nanoparticle amplification based quartz crystal microbalance DNA sensor for detection of Escherichia coli O157:H7, Biosens. Bioelectron. 21 (2006) 1178–1185, https://doi.org/10.1016/j.bios.2005.04.021.

[45] L. Yang, Y. Li, Simultaneous detection of Escherichia coli O157:H7 and Salmonella Typhimurium using quantum dots as fluorescence labels, Analyst 131 (2006) 394–401, https://doi.org/10.1039/b510888h.

[46] Q. Guo, J.J. Han, S. Shan, D.F. Liu, S.S. Wu, Y.H. Xiong, W.H. Lai, DNA-based hybridization chain reaction and biotin–streptavidin signal amplification for sensitive detection of Escherichia coli O157:H7 through ELISA, Biosens. Bioelectron. 86 (2016) 990–995, https://doi.org/10.1016/j.bios.2016.07.049.

[47] F. Ma, A. Rehman, H. Liu, J. Zhang, S. Zhu, X. Zeng, Glycosylation of quinone-fused polythiophene for reagentless and label-free detection of E. coli, Anal. Chem. 87 (2015) 1560–1568, https://doi.org/10.1021/ac502712q.

[48] R. Ye, C. Zhu, Y. Song, J. Song, S. Fu, Q. Lu, X. Yang, M.J. Zhu, D. Du, H. Li, Y. Lin, One-pot bioinspired synthesis of all-inclusive protein-protein nanoflowers for point-of-care bioassay: detection of: E. coli O157:H7 from milk, Nanoscale 8 (2016) 18980–18986, https://doi.org/10.1039/c6nr06870g.

[49] X. Sun, Y. Wang, L. Zhang, S. Liu, M. Zhang, J. Wang, B. Ning, Y. Peng, J. He, Y. Hu, Z. Gao, CRISPR-Cas9 triggered two-step isothermal amplification method for E. coli O157:H7 detection based on a metal-organic framework platform, Anal. Chem. 92 (2020) 3032–3041, https://doi.org/10.1021/acs.analchem.9b04162.

[50] F. Rousset, L. Cui, E. Siouve, C. Becavin, F. Depardieu, D. Bikard, Genome-wide CRISPR-dCas9 screens in E. coli identify essential genes and phage host factors, PLoS Genet. 14 (2018), https://doi.org/10.1371/journal.pgen.1007749.

[51] L. Wang, X. Shen, T. Wang, P. Chen, N. Qi, B.C. Yin, B.C. Ye, A lateral flow strip combined with Cas9 nickase-triggered amplification reaction for dual food-borne pathogen detection, Biosens. Bioelectron. 165 (2020), https://doi.org/10.1016/j.bios.2020.112364.

[52] R.L. Scharff, Economic burden from health losses due to foodborne illness in the United States, J. Food Protect. 75 (2012) 123–131, https://doi.org/10.4315/0362-028X.JFP-11-058.

[53] C.P. Thompson, A.N. Doak, N. Amirani, E.A. Schroeder, J. Wright, S. Kariyawasam, R. Lamendella, N.W. Shariat, High-resolution identification of multiple Salmonella serovars in a single sample by using CRISPRSeroSeq, Appl. Environ. Microbiol. 84 (2018), https://doi.org/10.1128/AEM.00859-18.

[54] E.J. Threlfall, Antimicrobial drug resistance in Salmonella: problems and perspectives in food- and water-borne infections, FEMS Microbiol. Rev. 26 (2002) 141–148, https://doi.org/10.1016/S0168-6445(02)00092-X.

[55] Y. Shen, L. Xu, Y. Li, Biosensors for rapid detection of Salmonella in food: a review, Compr. Rev. Food Sci. Food Saf. 20 (2021) 149–197, https://doi.org/10.1111/1541-4337.12662.

[56] W.F. Fricke, M.K. Mammel, P.F. McDermott, C. Tartera, D.G. White, J.E. LeClerc, J. Ravel, T.A. Cebula, Comparative genomics of 28 Salmonella enterica isolates: evidence for crispr-mediated adaptive sublineage evolution, J. Bacteriol. 193 (2011) 3556–3568, https://doi.org/10.1128/JB.00297-11.

[57] D.F.M. Monte, M.A. Nethery, R. Barrangou, M. Landgraf, P.J. Fedorka-Cray, Whole-genome sequencing analysis and CRISPR genotyping of rare antibiotic-resistant Salmonella enterica serovars isolated from food and related sources, Food Microbiol. 93 (2021), https://doi.org/10.1016/j.fm.2020.103601.

[58] N. Shariat, R.E. Timme, J.B. Pettengill, R. Barrangou, E.G. Dudley, Characterization and evolution of Salmonella CRISPR-Cas systems, Microbiol. 161 (2015) 374–386, https://doi.org/10.1099/mic.0.000005.

[59] J. Shen, X. Zhou, Y. Shan, H. Yue, R. Huang, J. Hu, D. Xing, Sensitive detection of a bacterial pathogen using allosteric probe-initiated catalysis and CRISPR-Cas13a amplification reaction, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-019-14135-9.

[60] J.M. Farber, J.Z. Losos, Listeria monocytogenes: a foodborne pathogen, CMAJ (Can. Med. Assoc. J.) 138 (1988) 413–418, https://doi.org/10.1128/mr.55.4.752-752.1991.

[61] S. Lomonaco, D. Nucera, V. Filipello, The evolution and epidemiology of Listeria monocytogenes in Europe and the United States, Infect. Genet. Evol. 35 (2015) 172–183, https://doi.org/10.1016/j.meegid.2015.08.008.

[62] S. Meile, A. Sarbach, J. Du, M. Schuppler, C. Saez, M.J. Loessner, S. Kilcher, Engineered reporter phages for rapid bioluminescence-based detection and differentiation of viable Listeria cells, Appl. Environ. Microbiol. 86 (2020), https://doi.org/10.1128/AEM.00442-20.

[63] N. Shariat, E.G. Dudley, CRISPRs: molecular signatures used for pathogen subtyping, Appl. Environ. Microbiol. 80 (2014) 430–439, https://doi.org/10.1128/AEM.02790-13.

[64] S. Mehata, G.C. Duan, Molecular mechanism of multi-drug resistance in Shigella isolates from rural China, Nepal Med. Coll. J. 13 (2011) 27–29.

[65] M. Puzari, M. Sharma, P. Chetia, Emergence of antibiotic resistant Shigella species: a matter of concern, J. Infect. Publ. Health 11 (2018) 451–454, https://doi.org/10.1016/j.jiph.2017.09.025.

[66] P.J. Sansonetti, Shigellosis: an old disease in new clothes? PLoS Med. 3 (2006) 1465–1466, https://doi.org/10.1371/journal.pmed.0030354.

[67] S. Hussen, G. Mulatu, Z. Yohannes Kassa, Prevalence of Shigella species and its drug resistance pattern in Ethiopia: a systematic review and meta-analysis, Ann. Clin. Microbiol. Antimicrob. 18 (2019), https://doi.org/10.1186/s12941-019-0321-1.

[68] S. Chen, H. Liu, W. Liang, L. Hong, Insert sequences of CRISPR/Cas system regulate horizontal antibiotic gene transfer in Shigella, Int. J. Antimicrob. Agents (2018) https://www.sciencedirect.com/science/article/pii/S0924857918302796?dgcid=rss_sd_all.

[69] N. Elahi, M.H. Baghersad, M. Kamali, Precise, direct, and rapid detection of Shigella Spa gene by a novel unmodified AuNPs-based optical genosensing system, J. Microbiol. Methods 162 (2019) 42–49, https://doi.org/10.1016/j.mimet.2019.05.007.

[70] E. Stout, T. Klaenhammer, R. Barrangou, CRISPR-cas technologies and applications in food bacteria, Annu. Rev. Food Sci. Technol. 8 (2017) 413–437, https://doi.org/10.1146/annurev-food-072816-024723.

[71] K. Selle, R. Barrangou, Harnessing CRISPR-Cas systems for bacterial genome editing, Trends Microbiol. 23 (2015) 225–232, https://doi.org/10.1016/j.tim.2015.01.008.

[72] S. Sieuwerts, F.A.M. De Bok, J. Hugenholtz, J.E.T. Van Hylckama Vlieg, Unraveling microbial interactions in food fermentations: from classical to genomics approaches, Appl. Environ. Microbiol. 74 (2008) 4997–5007, https://doi.org/10.1128/AEM.00113-08.

[73] A.A. Gomaa, H.E. Klumpe, M.L. Luo, K. Selle, R. Barrangou, C.L. Beisel, Programmable removal of bacterial strains by use of genome- targeting CRISPR-cas systems, mBio 5 (2014), https://doi.org/10.1128/mBio.00928-13.

[74] D. Bikard, C. Euler, W. Jiang, P.M. Nussenzweig, G.G. W, X. Duportet, V.A. Fischetti, L.A. Marraffini, Development of sequence-specific antimicrobials based on programmable CRISPR-Cas nucleases, Nat. Biotechnol. 32 (2014) 1146–1150.

[75] R.J. Citorik, M. Mimee, T.K. Lu, Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases, Nat. Biotechnol. 32 (2014) 1141–1145, https://doi.org/10.1038/nbt.3011.

[76] R.A. Perera, P. Wang, M.R. Gomaa, R. El-Shesheny, A. Kandeil, O. Bagato, L.Y. Siu, M.M. Shehata, A.S. Kayed, Y. Moatasim, M. Li, L.L. Poon, Y. Guan, R.J. Webby, M.A. Ali, J.S. Peiris, G. Kayali, Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, june 2013, Euro Surveill. 18 (2013), https://doi.org/10.2807/1560-7917.ES2013.18.36.20574.

[77] D. Bikard, C.W. Euler, W. Jiang, P.M. Nussenzweig, G.W. Goldberg, X. Duportet, V.A. Fischetti, L.A. Marraffini, Exploiting CRISPR-cas nucleases to produce sequence-specific antimicrobials, Nat. Biotechnol. 32 (2014) 1146–1150, https://doi.org/10.1038/nbt.3043.

[78] L. Cui, A. Vigouroux, F. Rousset, H. Varet, V. Khanna, D. Bikard, A CRISPRi screen in E. coli reveals sequence-specific toxicity of dCas9, Nat. Commun. 9 (2018), https://doi.org/10.1038/s41467-018-04209-5.

[79] Ü. Pul, R. Wurm, Z. Arslan, R. Geißen, N. Hofmann, R. Wagner, Identification and characterization of E. coli CRISPR-cas promoters and their silencing by H-NS, Mol. Microbiol. 75 (2010) 1495–1512, https://doi.org/10.1111/j.1365-2958.2010.07073.x.

[80] J.E. Samson, S. Moineau, Bacteriophages in food fermentations: new frontiers in a continuous arms race, Annu. Rev. Food Sci. Technol. 4 (2013) 347–368, https://doi.org/10.1146/annurev-food-030212-182541.

[81] R.H. Baltz, Bacteriophage-resistant industrial fermentation strains: from the cradle to CRISPR/Cas9, J. Ind. Microbiol. Biotechnol. 45 (2018) 1003–1006, https://doi.org/10.1007/s10295-018-2079-4.

[82] S. Rimantas, G. Giedrius, F. Christophe, B. Rodolphe, H. Philippe, S. Virginijus, The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli, Nucleic Acids Res. 39 (2011) 9275–9282.

[83] E. Garneau Josiane, D. Marie-Ève, V. Manuela, A. Romero Dennis, B. Rodolphe, B. Patrick, F. Christophe, H. Philippe, H. Magadán Alfonso, M. Sylvain, The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA, Nature 468 (2010) 67.

[84] L.A. Marraffini, E.J. Sontheimer, CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA, Science 322 (80-) (2008) 1843–1845, https://doi.org/10.1126/science.1165771.

[85] R. Edgar, U. Qimron, The Escherichia coli CRISPR system protects from λ lysogenization, lysogens, and prophage induction, J. Bacteriol. 192 (2010) 6291–6294, https://doi.org/10.1128/JB.00644-10.

[86] T. Nozawa, N. Furukawa, C. Aikawa, T. Watanabe, B. Haobam, K. Kurokawa, F. Maruyama, I. Nakagawa, CRISPR inhibition of prophage acquisition in streptococcus pyogenes, PLoS One 6 (2011), https://doi.org/10.1371/journal.pone.0019543.

[87] K.L. Palmer, M.S. Gilmore, Multidrug-resistant enterococci lack CRISPR-cas, mBio 1 (2010), https://doi.org/10.1128/mBio.00227-10.

[88] R. Barrangou, E.G. Dudley, CRISPR-based typing and next-generation tracking technologies, Annu. Rev. Food Sci. Technol. 7 (2016) 395–411, https://doi.org/10.1146/annurev-food-022814-015729.

[89] R. Barrangou, P. Horvath, CRISPR: new horizons in phage resistance and strain identification, Annu. Rev. Food Sci. Technol. 3 (2012) 143–162, https://doi.org/10.1146/annurev-food-022811-101134.

[90] G. Gasiunas, R. Barrangou, P. Horvath, V. Siksnys, Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria, Proc. Natl. Acad. Sci. U. S. A 109 (2012), https://doi.org/10.1073/pnas.1208507109.

[91] P. Horvath, D.A. Romero, A. Cou, M. Richards, S. Moineau, P. Boyaval, C. Fremaux, R. Barrangou, Diversity , activity , and evolution of CRISPR loci in, J. Biotechnol. 190 (2008) 1401–1412.

[92] T. Smokvina, M. Wels, J. Polka, C. Chervaux, S. Brisse, J. Boekhorst, J.E.T.H. van Vlieg, R.J. Siezen, Lactobacillus paracasei comparative genomics: towards species pan-genome definition and exploitation of diversity, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0068731.

[93] F.P. Douillard, A. Ribbera, R. Kant, T.E. Pietilä, H.M. Järvinen, M. Messing, C.L. Randazzo, L. Paulin, P. Laine, J. Ritari, C. Caggia, T. Lähteinen, S.J.J. Brouns, R. Satokari, I. von Ossowski, J. Reunanen, A. Palva, W.M. de Vos, Comparative genomic and functional analysis of 100 Lactobacillus rhamnosus strains and their comparison with strain GG, PLoS Genet. 9 (2013), https://doi.org/10.1371/journal.pgen.1003683.

[94] A. Natsch, R. Emter, R. Badertscher, G. Brunner, T. Granier, S. Kern, G. Ellis, Response to the Letter to the Editor Regarding Our Article (Natsch et al., 2015), Chem. Res. Toxicol. 28 (2015) 2082–2084, https://doi.org/10.1021/acs.chemrestox.5b00423.

[95] R. Sanozky-Dawes, K. Selle, S. O'Flaherty, T. Klaenhammer, R. Barrangou, Occurrence and activity of a type II CRISPR-Cas system in Lactobacillus gasseri, Microbiol. 161 (2015) 1752–1761, https://doi.org/10.1099/mic.0.000129.

[96] A.E. Briner, R. Barrangou, Lactobacillus buchneri genotyping on the basis of clustered regularly interspaced short palindromic repeat (CRISPR) locus diversity, Appl. Environ. Microbiol. 80 (2014) 994–1001, https://doi.org/10.1128/AEM.03015-13.

[97] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J.A. Doudna, E. Charpentier, A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Science 337 (80-) (2012) 816–821, https://doi.org/10.1126/science.1225829.

[98] K. Selle, R. Barrangou, CRISPR-based technologies and the future of food science, J. Food Sci. 80 (2015) R2367–R2372, https://doi.org/10.1111/1750-3841.13094.

[99] R. Barrangou, J.P. van Pijkeren, Exploiting CRISPR-Cas immune systems for genome editing in bacteria, Curr. Opin. Biotechnol. 37 (2016) 61–68, https://doi.org/10.1016/j.copbio.2015.10.003.

[100] J.H. Oh, J.P. Van Pijkeren, CRISPR-Cas9-assisted recombineering in Lactobacillus reuteri, Nucleic Acids Res. 42 (2014), https://doi.org/10.1093/nar/gku623.

[101] L.A.M. Wenyan Jiang, David Bikard, David Cox, Feng Zhang, CRISPR-assisted editing of bacterial genomes, Nat. Biotechnol. 31 (2013) 233–239, https://doi.org/10.1038/nbt.2508.CRISPR-assisted.

[102] P. Kumari, P.K. Panda, E. Jha, N. Pramanik, K. Nisha, K. Kumari, N. Soni, M.A. Mallick, S.K. Verma, Molecular insight in to in vitro biocompatibility of phytofabricated copper oxide nanoparticles with human embryonic kidney cells, Nanomedicine 13 (2018) 2415–2433, https://doi.org/10.2217/nnm-2018-0175.

[103] R. Sheel, P. Kumari, P.K. Panda, M.D. Jawed Ansari, P. Patel, S. Singh, B. Kumari, B. Sarkar, M.A. Mallick, S.K. Verma, Molecular intrinsic proximal interaction infer oxidative stress and apoptosis modulated in vivo biocompatibility of P.niruri contrived antibacterial iron oxide nanoparticles with zebrafish, Environ. Pollut. 267 (2020), https://doi.org/10.1016/j.envpol.2020.115482.

[104] P. Umaraw, P.E.S. Munekata, A.K. Verma, F.J. Barba, V.P. Singh, P. Kumar, J.M. Lorenzo, Edible films/coating with tailored properties for active packaging of meat, fish and derived products, Trends Food Sci. Technol. 98 (2020) 10–24, https://doi.org/10.1016/j.tifs.2020.01.032.

[105] B. Sarkar, S.K. Verma, J. Akhtar, S.P. Netam, S.K. Gupta, P.K. Panda, K. Mukherjee, Molecular aspect of silver nanoparticles regulated embryonic development in Zebrafish (Danio rerio) by Oct-4 expression, Chemosphere 206 (2018) 560–567, https://doi.org/10.1016/j.chemosphere.2018.05.018.

[106] P. Kaur, R. Thakur, S. Kumar, N. Dilbaghi, Interaction of ZnO nanoparticles with food borne pathogens Escherichia coli DH5α and Staphylococcus aureus 5021 & their bactericidal efficacy, AIP Conf. Proc. 1393 (2011) 153–154, https://doi.org/10.1063/1.3653655.

[107] B.K. Das, S.K. Verma, T. Das, P.K. Panda, K. Parashar, M. Suar, S.K.S. Parashar, Altered electrical properties with controlled copper doping in ZnO nanoparticles infers their cytotoxicity in macrophages by ROS induction and apoptosis, Chem. Biol. Interact. 297 (2019) 141–154, https://doi.org/10.1016/j.cbi.2018.11.004.

[108] E.M. Petrus, S. Tinakumari, L.C. Chai, A. Ubong, R. Tunung, N. Elexson, L.F. Chai, R. Son, A study on the minimum inhibitory concentration and minimum bactericidal concentration of nano colloidal silver on food-borne pathogens, Int. Food Res. J. 18 (2011) 55–66.

[109] D.P. Tamboli, D.S. Lee, Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram negative bacteria, J. Hazard Mater. 260 (2013) 878–884, https://doi.org/10.1016/j.jhazmat.2013.06.003.

[110] M. Ruiz-Rico, É. Pérez-Esteve, C. de la Torre, A.I. Jiménez-Belenguer, A. Quiles, M.D. Marcos, R. Martínez-Máñez, J.M. Barat, Improving the antimicrobial power of low-effective antimicrobial molecules through nanotechnology, J. Food Sci. 83 (2018) 2140–2147, https://doi.org/10.1111/1750-3841.14211.

[111] J. de Almeida Roger, M. Magro, E. Spagnolo, E. Bonaiuto, D. Baratella, L. Fasolato, F. Vianello, Antimicrobial and magnetically removable tannic acid nanocarrier: a processing aid for Listeria monocytogenes treatment for food industry

applications, Food Chem. 267 (2018) 430–436, https://doi.org/10.1016/j.foodchem.2017.06.109.

[112] S. Kumari, P. kumari, P.K. Panda, N. Pramanik, S.K. Verma, M.A. Mallick, Molecular aspect of phytofabrication of gold nanoparticle from Andrographis peniculata photosystem II and their in vivo biological effect on embryonic zebrafish (Danio rerio), Environ. Nanotechnol. Monit. Manag. 11 (2019), https://doi.org/10.1016/j.enmm.2018.100201.

[113] R. Salomoni, P. Léo, A.F. Montemor, B.G. Rinaldi, M.F.A. Rodrigues, Antibacterial effect of silver nanoparticles in Pseudomonas aeruginosa, Nanotechnol. Sci. Appl. 10 (2017) 115–121, https://doi.org/10.2147/NSA.S133415.

[114] S. Shamaila, N. Zafar, S. Riaz, R. Sharif, J. Nazir, S. Naseem, Gold nanoparticles: an efficient antimicrobial agent against enteric bacterial human pathogen, Nanomaterials 6 (2016), https://doi.org/10.3390/nano6040071.

[115] Y. Qing, L. Cheng, R. Li, G. Liu, Y. Zhang, X. Tang, J. Wang, H. Liu, Y. Qin, Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies, Int. J. Nanomed. 13 (2018) 3311–3327, https://doi.org/10.2147/IJN.S165125.

[116] O. Chlumsky, S. Purkrtova, H. Michova, V. Svarcova, P. Slepicka, D. Fajstavr, P. Ulbrich, K. Demnerova, The effect of gold and silver nanoparticles, chitosan and their combinations on bacterial biofilms of food-borne pathogens, Biofouling 36 (2020) 222–233, https://doi.org/10.1080/08927014.2020.1751132.

[117] K.S. Uma Suganya, K. Govindaraju, V. Ganesh Kumar, T. Stalin Dhas, V. Karthick, G. Singaravelu, M. Elanchezhiyan, Blue green alga mediated synthesis of gold nanoparticles and its antibacterial efficacy against Gram positive organisms, Mater. Sci. Eng. C 47 (2015) 351–356, https://doi.org/10.1016/j.msec.2014.11.043.

[118] S.K. Verma, E. Jha, P.K. Panda, P. Kumari, N. Pramanik, S. Kumari, A. Thirumurugan, Molecular investigation to RNA and protein based interaction induced in vivo biocompatibility of phytofabricated AuNP with embryonic zebrafish, Artif. Cells, Nanomed. Biotechnol. 46 (2018) S671–S684, https://doi.org/10.1080/21691401.2018.1505746.

[119] E. Sawosz, A. Chwalibog, J. Szeliga, F. Sawosz, M. Grodzik, M. Rupiewicz, T. Niemiec, K. Kacprzyk, Visualization of gold and platinum nanoparticles interacting with Salmonella Enteritidis and Listeria monocytogenes, Int. J. Nanomed. 5 (2010) 631–637, https://doi.org/10.2147/IJN.S12361.

[120] C. Lu, L. Tang, F. Gao, Y. Li, J. Liu, J. Zheng, DNA-encoded bimetallic Au-Pt dumbbell nanozyme for high-performance detection and eradication of Escherichia coli O157:H7, Biosens. Bioelectron. 187 (2021), https://doi.org/10.1016/j.bios.2021.113327.

[121] C. Cui, L. Gan, H.H. Li, S.H. Yu, M. Heggen, P. Strasser, Octahedral PtNi nanoparticle catalysts: exceptional oxygen reduction activity by tuning the alloy particle surface composition, Nano Lett. 12 (2012) 5885–5889, https://doi.org/10.1021/nl3032795.

[122] T. Niaz, S. Shabbir, T. Noor, M. Imran, Antimicrobial and antibiofilm potential of bacteriocin loaded nano-vesicles functionalized with rhamnolipids against foodborne pathogens, LWT (Lebensm.-Wiss. & Technol.) 116 (2019), https://doi.org/10.1016/j.lwt.2019.108583.

[123] R. Calderón-Gonzalez, H. Terán-Navarro, E. Frande-Cabanes, E. Ferrández-Fernández, J. Freire, S. Penadés, M. Marradi, I. García, J. Gomez-Román, S. Yañez-Díaz, C. Álvarez-Domínguez, Pregnancy vaccination with gold glyco-nanoparticles carrying Listeria monocytogenes peptides protects against listeriosis and brain- and cutaneous-associated morbidities, Nanomaterials 6 (2016), https://doi.org/10.3390/nano6080151.

[124] M. Varshney, L. Yang, X.L. Su, Y. Li, Magnetic nanoparticle-antibody conjugates for the separation of Escherichia coli O157:H7 in ground beef, J. Food Protect. 68 (2005) 1804–1811, https://doi.org/10.4315/0362-028X-68.9.1804.

[125] T. Elkin, X. Jiang, S. Taylor, Y. Lin, L. Gu, H. Yang, J. Brown, S. Collins, Y.P. Sun, Immuno-carbon nanotubes and recognition of pathogens, Chembiochem 6 (2005) 640–643, https://doi.org/10.1002/cbic.200400337.

[126] Y. Lin, X. Jiang, T. Elkin, K.A.S. Fernando, L. Gu, S. Taylor, H. Yang, E. Jones, W. Wang, Y.P. Sun, Carbon nanotubes for immunomagnetic separation of escherichia Coli O157:H7, J. Nanosci. Nanotechnol. 6 (2006) 868–871, https://doi.org/10.1166/jnn.2006.130.

[127] L. Gu, T. Elkin, X. Jiang, H. Li, Y. Lin, L. Qu, T.R.J. Tzeng, R. Joseph, Y.P. Sun, Single-walled carbon nanotubes displaying multivalent ligands for capturing pathogens, Chem. Commun. (2005) 874–876, https://doi.org/10.1039/b415015e.

[128] X. Chen, A. Miller, S. Cao, Y. Gan, J. Zhang, Q. He, R.-Q. Wang, X. Yong, P. Qin, B.H. Lapizco-Encinas, K. Du, Rapid Escherichia coli trapping and retrieval from bodily fluids via a three-dimensional bead-stacked nanodevice, ACS Appl. Mater. Interfaces 12 (2020) 7888–7896, https://doi.org/10.1021/acsami.9b19311.

[129] R. Jarquin, I. Hanning, S. Ahn, S.C. Ricke, Development of rapid detection and genetic characterization of Salmonella in poultry breeder feeds, Sensors 9 (2009) 5308–5323, https://doi.org/10.3390/s90705308.

[130] J. Hu, F. Tang, Y.Z. Jiang, C. Liu, Rapid screening and quantitative detection of: Salmonella using a quantum dot nanobead-based biosensor, Analyst 145 (2020) 2184–2190, https://doi.org/10.1039/d0an00035c.

[131] W. Jiang, D. Bikard, D. Cox, F. Zhang, L.A. Marraffini, RNA-guided editing of bacterial genomes using CRISPR-Cas systems, Nat. Biotechnol. 31 (2013) 233–239, https://doi.org/10.1038/nbt.2508.

[132] C. Bayraç, F. Eyidoğan, H. Avni Öktem, DNA aptamer-based colorimetric detection platform for Salmonella Enteritidis, Biosens. Bioelectron. 98 (2017) 22–28, https://doi.org/10.1016/j.bios.2017.06.029.

[133] M. Angelopoulou, K. Tzialla, A. Voulgari, M. Dikeoulia, I. Raptis, S.E. Kakabakos, P. Petrou, Rapid detection of salmonella typhimurium in drinking water by a

white light reflectance spectroscopy immunosensor, Sensors 21 (2021), https://doi.org/10.3390/s21082683.

[134] X. Dong, P. Wang, J.P. Darby, Y. Tang, C.M. Overton, S. Kathariou, Y.P. Sun, L. Yang, Photoactivated carbon dots for inactivation of foodborne pathogens listeria and salmonella, Appl. Environ. Microbiol. 87 (2021), https://doi.org/10.1128/AEM.01042-21.

[135] N. Elahi, M. Kamali, M.H. Baghersad, B. Amini, A fluorescence Nano-biosensors immobilization on Iron (MNPs) and gold (AuNPs) nanoparticles for detection of Shigella spp, Mater. Sci. Eng. C 105 (2019), https://doi.org/10.1016/j.msec.2019.110113.

[136] A.K. Dubey, V. Kumar Gupta, M. Kujawska, G. Orive, N.-Y. Kim, C. Li, Y. Kumar Mishra, A. Kaushik, Exploring nano-enabled CRISPR-Cas-powered strategies for efficient diagnostics and treatment of infectious diseases, J. Nanostruct. Chem. (2022), https://doi.org/10.1007/s40097-022-00472-7.

[137] Q.A. Phan, L.B. Truong, D. Medina-Cruz, C. Dincer, E. Mostafavi, CRISPR/Cas-powered nanobiosensors for diagnostics, Biosens. Bioelectron. 197 (2022), https://doi.org/10.1016/j.bios.2021.113732.

[138] O. Shalem, N.E. Sanjana, F. Zhang, High-throughput functional genomics using CRISPR-Cas9, Nat. Rev. Genet. 16 (2015) 299–311, https://doi.org/10.1038/nrg3899.

[139] D. Kim, Q.V. Le, Y. Wu, J. Park, Y.K. Oh, Nanovesicle-mediated delivery systems for crispr/cas genome editing, Pharmaceutics 12 (2020) 1–42, https://doi.org/10.3390/pharmaceutics12121233.

[140] Z. Wang, Y.H. Lee, B. Wu, A. Horst, Y. Kang, Y.J. Tang, D.R. Chen, Anti-microbial activities of aerosolized transition metal oxide nanoparticles, Chemosphere 80 (2010) 525–529, https://doi.org/10.1016/j.chemosphere.2010.04.047.

[141] E.M. Elmowafy, M. Tiboni, M.E. Soliman, Biocompatibility, biodegradation and biomedical applications of poly(lactic acid)/poly(lactic-co-glycolic acid) micro and nanoparticles, J. Pharm. Invest. 49 (2019) 347–380, https://doi.org/10.1007/s40005-019-00439-x.

[142] T. Taharabaru, R. Yokoyama, T. Higashi, A.F.A. Mohammed, M. Inoue, Y. Maeda, T. Niidome, R. Onodera, K. Motoyama, Genome editing in a wide area of the brain using dendrimer-based ternary polyplexes of Cas9 ribonucleoprotein, ACS Appl. Mater. Interfaces 12 (2020) 21386–21397, https://doi.org/10.1021/acsami.9b21667.

[143] H.J. Kim, S. Ogura, T. Otabe, R. Kamegawa, M. Sato, K. Kataoka, K. Miyata, Fine-tuning of hydrophobicity in amphiphilic polyaspartamide derivatives for rapid and transient expression of messenger RNA directed toward genome engineering in brain, ACS Cent. Sci. 5 (2019) 1866–1875, https://doi.org/10.1021/acscentsci.9b00843.

[144] H. Huang, C. Chai, N. Li, P. Rowe, N.P. Minton, S. Yang, W. Jiang, Y. Gu, CRISPR/Cas9-Based efficient genome editing in Clostridium ljungdahlii, an autotrophic gas-fermenting bacterium, ACS Synth. Biol. 5 (2016) 1355–1361, https://doi.org/10.1021/acssynbio.6b00044.

[145] Y. Gong, S. Tian, Y. Xuan, S. Zhang, Lipid and polymer mediated CRISPR/Cas9 gene editing, J. Mater. Chem. B. 8 (2020) 4369–4386, https://doi.org/10.1039/d0tb00207k.

[146] L. Bai, H. Zhou, R. Xu, Y. Zhao, K. Chinnaswamy, D. McEachern, J. Chen, C.Y. Yang, Z. Liu, M. Wang, L. Liu, H. Jiang, B. Wen, P. Kumar, J.L. Meagher, D. Sun, J.A. Stuckey, S. Wang, A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression in vivo, Cancer Cell 36 (2019), https://doi.org/10.1016/j.ccell.2019.10.002, 498-511.e17.

[147] A. Yamagishi, D. Matsumoto, Y. Kato, Y. Honda, M. Morikawa, F. Iwata, T. Kobayashi, C. Nakamura, Direct delivery of Cas9-sgRNA ribonucleoproteins into cells using a nanoneedle array, Appl. Sci. 9 (2019), https://doi.org/10.3390/app9050965.

[148] M. Hansen-Bruhn, B.E. de Ávila, M. Beltrán-Gastélum, J. Zhao, D.E. Ramírez-Herrera, P. Angsantikul, K. Vesterager Gothelf, L. Zhang, J. Wang, Active intracellular delivery of a cas9/sgRNA complex using ultrasound-propelled nanomotors, Angew. Chem. 130 (2018) 2687–2691, https://doi.org/10.1002/ange.201713082.

[149] S. Du, S.S. Liew, L. Li, S.Q. Yao, Bypassing endocytosis: direct cytosolic delivery of proteins, J. Am. Chem. Soc. 140 (2018) 15986–15996, https://doi.org/10.1021/jacs.8b06584.

[150] Y.K. Kang, K. Kwon, J.S. Ryu, H.N. Lee, C. Park, H.J. Chung, Nonviral genome editing based on a polymer-derivatized CRISPR nanocomplex for targeting bacterial pathogens and antibiotic resistance, Bioconjugate Chem. 28 (2017) 957–967, https://doi.org/10.1021/acs.bioconjchem.6b00676.

[151] B. Chopade, Patil Ghosh, Kitture Ahire, Kale Jabgunde, Cameotra Pardesi, Dhavale Bellare, Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents, Int. J. Nanomed. (2012) 483, https://doi.org/10.2147/ijn.s24793.

[152] S.K. Verma, E. Jha, P.K. Panda, J.K. Das, A. Thirumurugan, M. Suar, S. Parashar, Molecular aspects of core-shell intrinsic defect induced enhanced antibacterial activity of ZnO nanocrystals, Nanomedicine 13 (2017) 43–68, https://doi.org/10.2217/nnm-2017-0237.

[153] Y. Tu, M. Lv, P. Xiu, T. Huynh, M. Zhang, M. Castelli, Z. Liu, Q. Huang, C. Fan, H. Fang, R. Zhou, Destructive extraction of phospholipids from Escherichia coli

[154] C.P. Adams, K.A. Walker, S.O. Obare, K.M. Docherty, Size-dependent antimicrobial effects of novel palladium nanoparticles, PLoS One 9 (2014), https://doi.org/10.1371/journal.pone.0085981.

[155] X. Hong, J. Wen, X. Xiong, Y. Hu, Shape effect on the antibacterial activity of silver nanoparticles synthesized via a microwave-assisted method, Environ. Sci. Pollut. Res. 23 (2016) 4489–4497, https://doi.org/10.1007/s11356-015-5668-z.

[156] A. Abbaspour, F. Norouz-Sarvestani, A. Noori, N. Soltani, Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of staphylococcus aureus, Biosens. Bioelectron. 68 (2015) 149–155, https://doi.org/10.1016/j.bios.2014.12.040.

[157] H. Shen, J. Wang, H. Liu, Z. Li, F. Jiang, F.B. Wang, Q. Yuan, Rapid and selective detection of pathogenic bacteria in bloodstream infections with aptamer-based recognition, ACS Appl. Mater. Interfaces 8 (2016) 19371–19378, https://doi.org/10.1021/acsami.6b06671.

[158] D.M. Kim, J.S. Park, S.W. Jung, J. Yeom, S.M. Yoo, =Biosensing applications using nanostructure-based localized surface plasmon resonance sensors, Sensors 21 (2021), https://doi.org/10.3390/s21093191.

[159] M. Hu, C. Yuan, T. Tian, X. Wang, J. Sun, E. Xiong, X. Zhou, Single-step, salt-aging-free, and thiol-free freezing construction of AuNP-based bioprobes for advancing CRISPR-based diagnostics, J. Am. Chem. Soc. 142 (2020) 7506–7513, https://doi.org/10.1021/jacs.0c00217.

[160] J.H. Choi, J. Lim, M. Shin, S.H. Paek, J.W. Choi, CRISPR-Cas12a-Based nucleic acid amplification-free DNA biosensor via Au nanoparticle-assisted metal-enhanced fluorescence and colorimetric analysis, Nano Lett. 21 (2021) 693–699, https://doi.org/10.1021/acs.nanolett.0c04303.

[161] M. Hu, C. Yuan, T. Tian, X. Wang, J. Sun, E. Xiong, X. Zhou, Single-step, salt-aging-free, and thiol-free freezing construction of AuNP-based bioprobes for advancing CRISPR-based diagnostics, J. Am. Chem. Soc. 142 (2020) 7506–7513, https://doi.org/10.1021/jacs.0c00217.

[162] S.H. Nile, V. Baskar, D. Selvaraj, A. Nile, J. Xiao, G. Kai, Nanotechnologies in food science: applications, recent Trends, and future perspectives, Nano-Micro Lett. 12 (2020), https://doi.org/10.1007/s40820-020-0383-9.

[163] A. Ahmad, J.M. Khan, S. Haque, Strategies in the design of endosomolytic agents for facilitating endosomal escape in nanoparticles, Biochimie 160 (2019) 61–75, https://doi.org/10.1016/j.biochi.2019.02.012.

Abbreviation

MDR: Multidrug-resistant
WHO: World Health Organization
EFSA: European food Safety Authority
CDC: Centers for Disease Control and Prevention
AMR: Anti-microbial resistance
RBP: Ribosome Binding Protein
NPs: Nanoparticles
ROS: Reactive Oxygen Species
MRSA: Methicillin Resistant *Staphylococcus aureus*
CCB: CRISPR-Cas13a based
DEC: Diarrheagenic *E. coli*
CFU: Colony Forming Unit
NLuc: Nano-luciferase
WGS: Whole Genome Sequencing
LOD: Limit of Detection
LOQ: Limit of Quantitation
gRNA: Guide RNA
crRNA: CRISPR RNA
*tracrRNA: Trans-*activating CRISPR RNA
H– NS: Histone-like nucleoid structuring
MIC: Minimum Inhibitory Concentration
MBC: Minimum Bactericidal Concentration
AgNPs: Silver Nanoparticles
AuNPs: Gold Nanoparticles
Pt-NPs: Platimun Nanoparticles
SAMN@TA: Surface active maghemite NPs with tannic acid
RSNVs: Rhamnosome Nano-Vesicles
MNCs: Magnetic nanoparticle-antibody conjugates
MCE: Minimum Cpture Efficiency
SWNT: Single-walled nanotube
MWNT: Multi-walled nanotube
IMS: Immune-magnetic separation
MDA: Multiple displacement amplifcation
SNP: Single nucleotide polymorphism
SPCE: Screen-printed carbon electrode
DPV: Differential pulse voltammetry
MNPs: Magnetic Nanoparticles
sgRNA: Single guide RNA