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Functionalized Inorganic Nanoparticles for the Detection of Food and Waterborne Bacterial Pathogens

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Abstract Infections acquired from ingesting contaminated food and water poses an adverse effect on public health and safety, thus affecting nations' economy. Technical approaches developed over years have contributed adequately to microbial detection in food and water, yet, unveiling spaces for more improvement on early and rapid detection of pathogens. This review highlights different strategy assessing bio-functionalized inorganic nanoparticles towards the detection of pathogens in food and water samples. Conjugates of several bio-receptors and inorganic nanoparticles showed rapid, real-time, repeatability, and appreciable limit of detection in targeted pathogens. A patent referenced in this study established the biocompatibility of bio-functionalized inorganic nanoparticles mechanism. Unique attributes exhibited by bio-functionalized inorganic nanoparticles showed potential and improvement of the existing bio-sensing pathogen detection methods. Each of the identified strategies described showed a promising pathway accommodating the development of simple, and even the fabrication of low-cost materials for easy detection of bacterial pathogens in food and water products.

Keywords: *foodborne infections, waterborne infections, bacterial detection, functionalized inorganic nanoparticles, Bioreceptors*

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1. Food and Waterborne Pathogen and Its Effect

Pathogenic bacteria from time immemorial and presently are still of significant concern to human health and safety, as they cause deleterious changes to man healthy living [1]. Although they are ubiquitous, their presence in food and water poses more harmful health risk resulting in both mild and fatal diseases even at a low infectious dose [2]. Some common illnesses and leading causal agents associated with food and water contamination globally are acute gastroenteritis, food poisoning (*Staphylococcus aureus, Clostridium perfringens, Bacillus cereus*), Botulism (*Clostridium botulinum*), Campylobacteriosis (*Campylobacter jejuni*), Listeriosis (*Listeria monocytogenes*), Salmonellosis (*Salmonella spp*), Hemorrhagic colitis (*Escherichia coli* O157:H7), Cholera (*Vibrio cholera*), diarrhoea (*Vibrio parahaemolyticus*) among others [3,4,5].

Among the pathogens identified as causal agents in food- and waterborne illnesses, enteric pathogens namely; *Salmonella spp*, *Shigella spp*, *Yersinia enterolitica*, *Aeromonas spp* and pathogenic *Escherichia coli* have been incriminated as the major pathogens causing hospitalizations, and even deaths due to the production of toxins and other cell metabolites within their host [6,7]. In addition to the severity of infections experienced by humans, the financial burden of food and water-borne infections has hadsome negative impact on individual's income and nation's economy. Areas affected majorly include: increase in medical expenses, productivity loss and loss of human resources [8]. Its financial implication is estimated to cause a loss of about $15.5 billion in the US, and $110.2 billion in low- and middle-income countries each year [8,9]. The impact of waterborne disease has also been devastating, causing an economic loss of nearly $12 billion annually. The significant impact of this loss has been well felt in developing countries, as it expands their poverty rate and margin among its populace. As a means to preserve the public health, early detection and analysis of bacterial pathogens which could be life-threatening is quite essential [10]. These could be a landmark achievements in clinical medicine, agriculture, food safety, public health and biosecurity. Pathogen detection methods is classified into the following sections: conventional methods, mass spectroscopy, and sensor-based methods.

2. Conventional Methods for Pathogen Detection

The detection of pathogens using conventional methods depends mostly on strategies which involve precise identification based on microbiological, biochemical

(phenotypic) and molecular (genotypic) constituents displayed by the organisms [11]. Sub-methods described in most conventional methods include traditional-based methods, immunology-based methods and nucleic acid-based methods [12,13,14]. Traditional-based method otherwise termed culture-based avails several approaches for pathogen detection. This method depends on the culturing of microbes on agar plates, the most probable number, membrane filtration, and many more. They are a widely recognized approach for their low cost and ease of use. The culturing techniques are highly dependable, relatively interesting compared to other methods, and expressing results both qualitatively and quantitatively [15]. Qualitative traditional-based methods determine the presence or absence of pathogens in samples, while quantitative traditional-based methods are quite useful for enumeration.

Immunology-based method has been the most popular, successful and widely accepted technology in bacterial, spores, viruses and toxins detection, especially for Gram-negative bacteria. Several techniques associated with immunological detection include enzyme immunoassay (EIA), enzyme-linked fluorescent assay (ELFA) [16,17], enzyme-linked immunosorbent assay (ELISA) [18], flow injection immunoassay, and others. It is faster (require less time preparing assay than traditional-based methods), robust and possesses the ability to detect organisms responsible for the contamination. ELISA and lateral flow immunoassay are the most accepted immunological methods applicable in foodborne pathogen detection, as they are not laborious and a large number of samples can be analyzed [19,20].

In the use of molecular based methods for the detection of pathogens, several techniques embedded in the nucleic acid-based method includes polymerase chain reaction (PCR), 16S rRNA (Denaturing Gradient Gel Electrophoresis-DGGE, Restriction Fragment Length Polymorphism-RFLP, and Ribosomal RNA Intergenic Spacer Analysis-RISA), Fluorescence in-situ Hybridization-FISH, microarray, Sequencing, Next Generation Sequencing (NGS) and many more. These methods have proven its efficiency by preventing ambiguity as well as wrong interpretation of results. Due to their precision and accuracy, they become imperative and are categorized as conventional techniques for the detection of pathogens. The nucleic acid-based method showed high sensitivity and specificity as it involves the use of high-throughput techniques to identify microbes, address their sources and point of variations, and thus, revolutionizing approaches to the study of microbial and clonal diversity.

2.1. Mass Spectroscopy for Pathogen Detection

A new phase towards the detection of pathogens was achieved with the advent of Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). These techniques identify microorganisms based on the uniqueness of the mass protein spectral fingerprints present on their cell surfaces. It has been applied for the detection of pathogens protein marker signals in different samples [21]. One significant advantage of MALDI-TOF MS is the dynamism of its proteome profiling property which enables the addition of new microorganism's protein spectral into the database [22]. However, few setbacks in this method include the need for enrichment step before a test, the availability of the peptide mass fingerprints in the database which must correspond and identify tested microorganisms, high cost of procurement and setting-up.

2.2. Limitations of the Established Pathogen Detection Methods

In spite of the benefits derived from each method, some major setbacks limiting its adequacy for point and quick detection approach has not been pleasing well enough. Consideration of the conventional methods, shows they are laborious -taking 2-3 days and 6-7 days often before initial results and specific pathogens are determined respectively. In some cases, the futility of normal culture plate technique often requires pre-enrichment, selective enrichment, selective plating and identifications. The sequence of the method is stereotyped, lengthy, time-consuming, limited by its low sensitivity -giving false-negative results even with the presence of viable but non-culturable (VBNC) cells [23,24]. Immunology-based methods present varying disadvantages such as the use of expensive test kits, false result due to cross-reactivity with closely related antigens, the need for pre-enrichment to attain detectable antigen level in samples. Major difficulties are also often encountered when expert staff default in engaging proper laboratory procedures which include proper labelling of antigens and antibodies, and accommodating external interference that should be limited. Its dependence on the amount of antigen in the sample which determines the specific binding response from the antibodies is also of major concern [25].

Furthermore, PCR methods -a highly predictive method still requires the use of expensive instruments for its nucleic acid amplification and quantification. Other complexities of PCR are the necessities for specific primers after the microorganisms' subjection to probable cultural identification, its optimal reaction mixture which requires trained personnel to operate and avoid risk of false positive and negative results. Sensor-based method devised for pathogenic substances detection without special sample pre-enrichment, is still limited due to the difficulty experienced in the enhancement of immobilized bio-components stability. Its high cost in instrumentation design and quality assurance slows its commercial and laboratory methods than other rapid methods [25]. These setbacks highlighted above creates the need to search and re-evaluate existing methods for newer approaches affordable and efficient for pathogen detection.

3. Sensor-Based Methods for Pathogen Detection

Sensor-based methods have been proven to be a dependable device useful for the detection of living organisms, biological molecules as well as to detect chemical components present in living organisms. Recently, research activities have increasingly drifted towards the use of the sensor-based approach for pathogens detection. They have become essential in pathogens and toxins

detection, environmental monitoring [26], soil quality monitoring, drug discovery, prosthetic devices [25], timely detection of post-harvest deterioration [27], and food quality monitoring [28]. The wide applications are as a result of its short-time analysis, portability in the design of biological analytical techniques, exemption of sample pre-enrichment approach, its efficiency in real-time measurements and automation [11]. The use of a sensor-based method has become a favorable method to ensure food safety both in real-time and during the production process [29]. Food industries are currently engaged in the development/use of bio-recognition elements for pathogen detection in products. Sensor-based methods which had gained rapid technological growth is constituted of two major materials namely the bio-receptor (biological capture molecule/biosensing or bio-recognition elements) and the transducer -which converts bio-recognized energy/ biological response into signals.

Bio-receptors are an important component in the development of sensor-based methods because their surfaces are specifically designed using some biochemical mechanisms to recognize and initiate binding with the analyzed materials. Varying bio-receptors reported for sensor-based method efficiency include antigens, antibodies, enzymes, nucleic acids, cells, microorganisms, aptamers, bio-mimic substance and many more as shown in Figure 1. Binding initiation by the bio-receptors to the analyzed materials of interest activates the reaction for sensor measurement via a transducer [30].

Optical, electrochemical, spectroscopy and magnetic capture/separation transducers were found to be widely used for pathogen detection. Similarly, some of the widely accepted transducers have also been reported to be used in combination with other less important ones for improved efficiency [31,32,33].

3.1. Functionalizing Nanoparticles for Pathogen Detection in Sensor-based Methods

Improving the sensitivity of bio-receptors for bacteria detection ushered acceptance in the use of nano-materials as signal amplifiers. Functionalizing nano-materials with bio-receptors showed a high level of success in the development of bacterial biosensors due to its good conductivity, high surface-to-volume ratio, diffusion rate and good result outputs on the transducers [5]. The feat is achievable because nanotechnology through its approach encourages bio-fabrication of useful devices from nano-materials to form conjugate macromolecules with other materials. Yang *et al.* [36] in their study on the subject established the advantages of incorporating nanoparticles in biosensor which includes rapid and real-time detection, ensure an improved detection sensitivity and enabling the detection of multiple pathogens simultaneously. Also, they are versatile, easy to manipulate, biocompatible enough to enhance signal effects when combined with varieties of biological, molecular and artificial/synthetic materials, and thus, produces a visible colorimetric result [5].

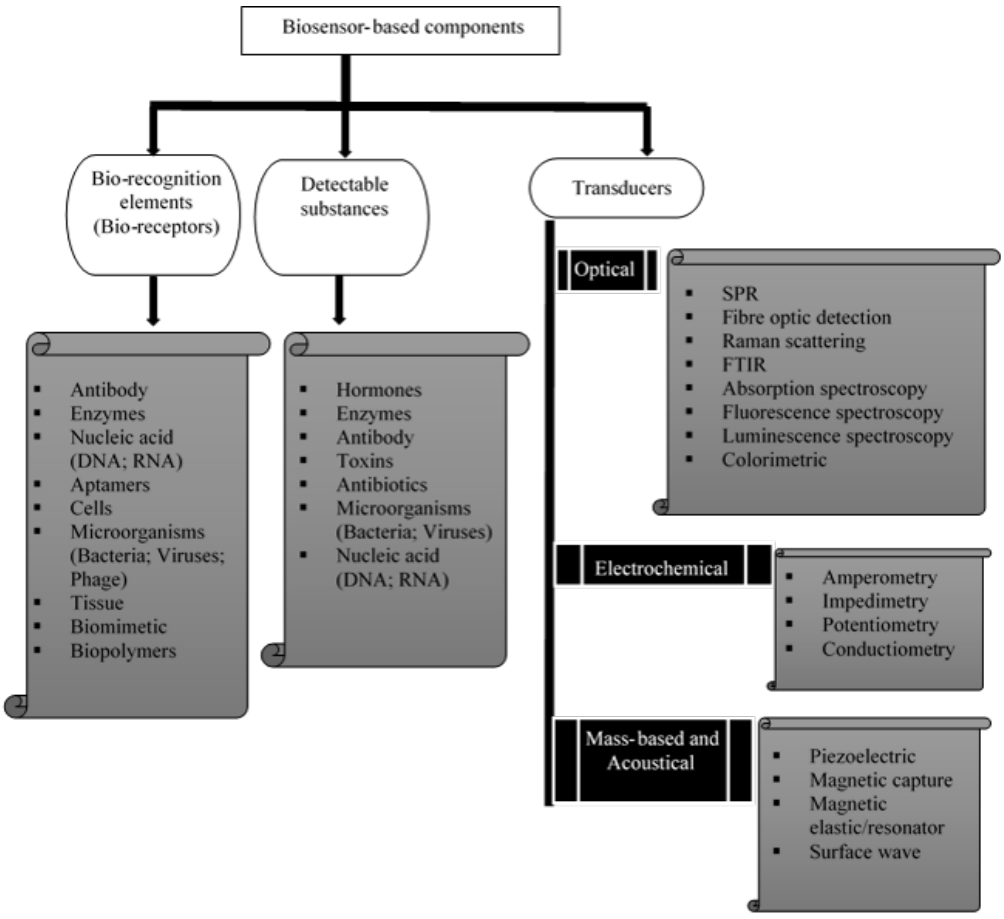

Figure 1. Essential components in Biosensor-based methods (Adapted from 19,34,35)

Surface modification and functionalizing inorganic nanoparticles (metal and semi-conductor) with bio-recognition elements have become a widespread practice with many attempts to improve and ensure its analytical sensitivity [37]. Metal and semi-conductor nanoparticles (Table 1) functionalized with materials provides amplified signals for the detection of bacteria [38]. For proper functionalization and modification, appropriate bio-receptors materials are required to provide active biocompatibility with analyzed materials, enhanced signals as well as rapid detection procedure [39]. Thus, in achieving selectivity and improve sensitivity, nano-materials needs to be interfaced with biological, molecular and artificial receptors for specific binding and target of bacteria [40].

Table 1. Forms of Inorganic Metallic Nanoparticles

Forms	Metals Involved	References
Pure Metals	Ag, Au, Cu, Fe, Ni, Co, Pt, Palladium	[41,42]
Metal oxides (Semi-conductor)	ZnO, CuO, TiO_2, CrO_2, SiO_2	[41,42]
Magnetic	Fe_3O_4, Fe_2O_4,	[42]
Metallic Chalcogenides	PbS, ZnS, CdS, FeS, HgS, ZnSe, CdTe, CuInSe	[42]
Bi-metallic	Ag-Au; Zn-Ag, Pt-Ni, Co-Mo, Ag-MgO, Fe_3O_4-Au, Fe_3O_4-Ag, $MnFe_2O_4$-Au, Fe-Pt, Fe_3O_4-Zn, Fe_3O_4-SiO_2	[2,42-46]

Advancement in nanotechnology and its approach towards the exploration of more sensitive optical biosensors had created more interest in the development of new nano-structured materials, especially metal for improved overall performance as bacterial biosensors [26,47,48]. Attachment of bio-receptors to the surfaces of nano-materials (Figure 2) have been either through a direct or indirect method. Direct method namely: physical adsorption or covalent coupling could both be exhibited by hydrophobic and electrostatic interactions. Polyethylene glycol (PEG), Poly-L-lysine (PLL), and Polyethylenimine (PEI) are typical examples of materials used. The indirect method forms a bridge link between biomolecules and nano-materials with corresponding high affinity with biotin, avidin, and streptavidin as typical examples of linkers [49]. According to Yang *et al.* [36], the conjugation of biomolecules with nano-materials is the foundation of nano-biorecognition. Based on the nature of biomolecules conjugated to nano-materials, there are antibody-antigen, adhesin-receptor, antibiotic-antigenic surface, and complementary DNA sequence recognitions.

3.1.1. Functionalized Pure Noble Metal Nanoparticles (Gold Nanoparticles)

A novel attempt which involved the use of monomeric sugar to label a protein present on the bacteria cell surface to aid microscopy view has been achieved. Lin *et al.* [50] reported functionalized gold nanoparticles (AuNPs) with monomeric mannose sugar specifically recognized adhesion FimH of type 1 pili in *Escherichia coli*. It has been documented that antibody tends to produce reliable affinity, increases surface area to volume ratio and also amplifies signals. The use of a specific antibody as functionalizing agent for AuNPs was reported by Basu *et al.* [51], with preferences given to the development of immunochromatographic strip (Figure 3) for the detection of *Salmonella typhi* [52,53]. Other work by Baccar *et al.* [54] reported the use of acid-thiol, amine-thiol coupled with antibody to surface-modified gold nano-materials. Pengsuk *et al.* [55] in their study established the detection of *Vibrio cholerae* 0139 in seafood using gold nanoparticles and specific antibodies.

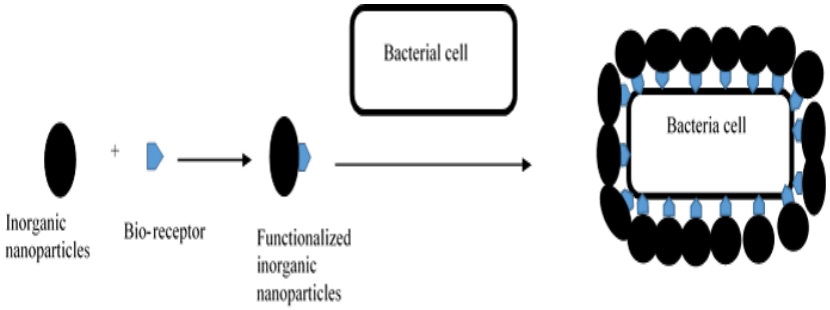

Figure 2. Overview of bacterial detection by functionalized inorganic nanoparticles

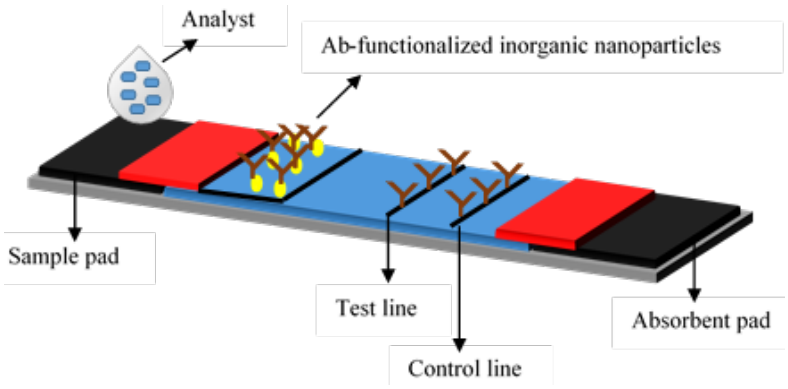

Figure 3. Components of an immunochromatographic strip

Augmenting antibody with other bio-receptors such as chitosan to serve as cross-linker effectively increases surface area for reactivity as a result of their mesh structure. The combined effect of antibody-chitosan carried out by Kang *et al.* [56] showed the limit of detection as 10 CFU/mL. Thiramanas and Laocharoensuk [57] showed the effectiveness of Polyethylenimine-AuNPs to electrostatically bind to the surfaces of *enterotoxigenic E. coli* (ETEC) and *S. aureus*. PEI increases and stabilizes the electrostatic interaction (direct method) between the positively charged gold nanoparticles and negatively charged components on bacteria cell surfaces. Findings by Raj *et al.* [58] and Huang *et al.* [59] showed the use of cysteine and 4-mercaptophenylboronic acid (4-MPBA) as a good functionalizing agent respectively. This direct method of modifying AuNPs showcase color responses which can be directly observed with the naked eye or through digital-camera-based red, green and blue (RGB) colour model analysis. Aggregation of DNA probes on the surface of AuNPs was reported for the detection of *Mycobacterium* sp. and *S. aureus* [60].

On the other hand, surface modification of AuNPs with aptamers gained lots of attention in the detection of *Salmonella typhi* and *Shigella flexneri* in research works [61,62,63,64,65]. The major advantage of Aptamers is in its ability to prevent AuNPs from NaCl-induced aggregation. It was found to increase electron transfer, electrochemical signal and ascertain detection within a short period (20-60 minutes). Few studies evaluated the bi-functional approaches of aptamer with other bio-receptors such as 4-mercaptobenzoic acid (4-MBA) [66], oligonucleotide probe [67], chitosan [68], 4-MBA and citrate [69]. Each complementing bio-receptors reported showed fast, sensitive, specificity and accurate SERS based sensors towards the detection of pathogens in samples. The incorporation of 4-MBA tags as shown in these studies specifically increases Raman signal. Varying forms of these bioreceptors are shown in Table 2.

3.1.2. Functionalized Magnetic Nanoparticles

Prior to the engagement of magnetic nanoparticles in pathogen detection, a simple approach to separating and concentrating charged targets in aqueous solutions has been provided [5]. Magnetic nanoparticles (MNPs) -a derivative of complex iron salts, have gained thorough studies and widespread applications especially for pathogen detection due to their stability, biocompatible and large surface-to-volume ratio aiding microbial cell wall adherence for effective separation [70,71]. Most importantly, they tend to become more useful and beneficial with their readiness to combine and improve detection platforms such as mass spectra, surface plasmon resonance, electrochemical, Raman spectra, fluorescent, and many more [72] as shown in Table 3. Ju [73] in his review expatiated the use of Fe_3O_4 nanoparticles-based hybridized materials to enhance peroxidase-like activity, and thus enabling the detection of bio-materials.

Lin *et al.* [74] evaluated the importance of vancomycin as an active functionalizing component for MNPs showing some level of compatibility with Gram-positive bacteria as it analyzes *S. aureus* and *S. saprophyticus*. Vancomycin inclusion limits MALDI-TOF MS false error as it reduces the interference of protein and metabolite signals in the mass spectra of Gram-positive bacteria. Vancomycin is a target antibiotic with high specificity for D-Ala-D-Ala moieties on Gram-positive cell walls. Several studies documented the use of varying binding materials to attach antibodies to the surfaces of MNPs to establish high adherence, biocompatibility and rapid trapping of targeted pathogens [75,76,77]. The inclusion of HRP to antibody functionalized MNPs by Mun and Choi [78], prevents false positive signals. The development of DNA aptamer-coated MNPs for pathogen detection showed a realistic approach by improving peroxidase-like activities of MNPs and thus increases signals. Surface enhancement of MNPs with aptamer as described by Park *et al.* [79] and Wang *et al.* [80] showed improvement in the detection sensitivity of *S. typhi* and *S. aureus*. The application of oligonucleotide probe as functionalizing bio-element of MNPs was addressed by Li *et al.* [81] to aid instant detection of their bacterial targets.

Different approach explored by Matta and Alocilja [82] maximizes carbohydrate ligands (glycan and cysteine-glycan) as the functionalizing component for the detection of *S. enteritidis*, *E. coli* O157:H7 and *B. cereus* in pasteurized milk. As earlier documented, non-covalent electrostatic interaction towards bacteria cell surface is due to the presence of hydroxyl, amino and hydrophobic regions present in carbohydrate ligands. Functional groups on ligands show high affinity for lipids, sugars and proteins present on bacteria cell surfaces [83,84]. Functionalized MNPs by urease enzyme hydrolysis urea, increases the pH value and enhances the binding strength of the complex [72]. Hydrolysis of urea by urease enzyme in this work report colorimetric detection of the pathogen as a result of the increase in pH and thus promote detectability on litmus dye. Recent research articles showed the potential of chitosan. Le *et al.* [85] suggested that protonated amine group on chitosan attracts efficient and stronger binding quality with the anionic components on bacteria surfaces (*E. coli* and *S. aureus*) in an acidic pH condition.

3.1.3. Functionalized Metal Oxides Nanoparticles

Studies have documented the potential of metal oxides surface modification for capturing and detection of pathogens [87,90]. Amongst metal oxides, silica oxides (SiO_2) enables high photo-stability and conjugation with biomolecules. Zhao *et al.* [86] and Wang *et al.* [87] in their study reported functionalizing silica nanoparticles (SiO_2NPs) surfaces with antibodies for the detection of *E. coli* O157:H7, *S. typhi*, and *S. aureus* (Table 4). It was shown that the procedure facilitates strong and specific antibody-antigen interaction and recognition. Study on titanium oxide (TiO_2) revealed it has a wide band gap semi-conductor and good stability [88,89] which shows its essentiality for sensor-based detection. Viter *et al.* [90] evaluated the importance of titanium oxide nanoparticles (TiO_2NPs) functionalized by antibody and deposited on glass substrates for the detection of *Salmonella*. In their research findings, antibody-TiO_2NPs conjugates exhibited intense photoluminescence at the visible range spectrum which was attributed to the strong electrostatic interaction between TiO_2NPs and the antibody protein.

Table 2. Some Bacteria Detected in Functionalized Gold Nanoparticles (AuNPs)

Target Organisms	Bioreceptor Used	Developed Mechanisms	Detection Methods	Limit of Detection (CFU/ml)	References
E. coli	Mannose	Coupling of monomeric mannose to gold nanoparticle (m-AuNps) to target adhesin FimH of type 1 pili of the target pathogen.	Microscopy (TEM)	NA	[50]
E. coli 0157:H7	Antibody	Immobilized Gold nanowire arrays (GNWA) bounded with antibody prepared on alumina template trapped target pathogen to form a sandwich with second antibody conjugated with phosphatase.	Electrochemical Impedance Spectroscopy (EIS)	10 cells/0.173 cm^2	[51]
E. coli K12; L. fermentum	Antibody	Acid-thiol modified gold substrate form a conjugated with polyclonal antibody gold nanoparticle.	Surface Plasmon Resonance (SPR)	10^4 & 10^3	[54]
Mycobacterium sp.	DNA Probes	DNA probe of enzyme ALP and detector probe coated on gold nanoparticles were immobilized on indium tin oxide (ITO) electrode-coated glass slides.	Electrochemical Impedance Spectroscopy (EIS)	1.25 ng/ml	[60]
S. typhi	Antibody	Antibody coated gold nanoparticles were clotted on nitrocellulose membrane to form an immunochromatographic strip.	Colorimetric	1.14 x 10^5	[52]
Salmonella; E. coli 0157:H7	Aptamer	Aggregation of aptamers on the surface of gold nanoparticles and signal amplification upon high salt conditions.	Colorimetric; Optical UV-Vis	10^5	[61]
B. cereus	Antibody-Chitosan	Monoclonal antibody-modified gold nanoparticles trapped target pathogen was cross-linked to chitosan immobilized on glassy carbon electrode.	Amperometric (Cyclic voltammetry)	10	[56]
S. typhi	Aptamers	Biotinylated aptamer immobilized on biotin and streptavidin-coated microtiter plate-wells trapped target pathogen, and sandwiched with aptamer-coated gold nanoparticles to complete the assay.	Colorimetric; UV-Vis Spectroscopy @ 630 nm	7	[62]
E. coli 0157:H7	Cysteine	Gold nanoparticles was modified with positively charged cysteine to accommodate electrostatic adhesion as a result of the different surface charges.	Colorimetric; Surface Plasmon Resonance (SPR); Microscopy (TEM)	100	[58]
ETEC; S. aureus	Polyethylenimine	Gold nanoparticles was coated with positively charged polyethyleneimine and the optical signal amplified using chlorophenol red β-D-galactopyranoside (CPRG).	Colorimetric	10	[57]
S. typhi	Aptamer	Gold nanoparticles surface was modified with aptamer and optical signal amplified upon high salt concentrations.	Colorimetric	56	[63]
S. typhi	Aptamer	Gold nanoparticles coated with thrombin binding aptamer were assembled on glass substrate surface.	Localized SPR	1.0 x 10^4	[64]
Streptococcus agalatiae	Monoclonal antibodies (4C12 & 3A9)	Conjugate of monoclonal antibody and Gold nanoparticles were assembled on the nitrocellulose membrane to form an immunochromatographic strip pad and over laid with capturing antibody.	Colorimetric	1.5 x 10^5	[53]
S. typhi	Aptamer	Biotinylated aptamer anchored on microtiter plate capture target pathogen and overlaid with 4-mercaptobenzoic acid-coated gold nanoparticles.	Surface Enhanced Raman Scattering (SERS) measurement	4	[66]
S. typhi	Oligonucleotide probe; Aptamer	Formation of Gold nanoparticles conjugated with bi-functional oligonucleotide probes and aptamer, and signal amplified upon NaCl solution addition	Colorimetric; UV-Vis Spectroscopy	10	[67]
S. flexneri	Aptamer	Gold nanoparticles coated with aptamers targets pathogen and the signal amplified upon NaCl solution addition.	Colorimetric; UV-Vis Spectroscopy @ 400-800nm; LAMP	80	[65]
S. typhi	Carboxymethyl chitosan; Aptamer	Composite comprising of carboxymethyl chitosan loaded with amino-modified aptamer functionalized gold nanoparticles to capture target pathogen.	Colorimetric; UV-Vis Spectroscopy @ 550nm	16	[68]
E. coli; Sal. Pullorum; S. aureus; Ent. Faecalis; S. mutans	4-mercaptophenylboronic acid	Gold nanoparticles modified with 4-MPBA aggregates on targeted pathogen surfaces.	Image capture (digital camera in black box); UV-Vis Spectroscopy @ 524nm	1.02 x 10^3	[59]
Shigella sonnei	Citrate; Aptamer	Citrate-stabilized Gold nanoparticles were conjugated with aptamer to target bacteria cells. Signal amplified with raman active 4-MBA ligand.	Surface Enhanced Raman Scattering (SERS) measurement	10	[69]

Table 3. Some Bacteria Detected in Functionalized Magnetic Nanoparticles (MNPs)

Target Organisms	Bioreceptor Used	Developed Mechanisms	Detection Methods	Limit of Detection (CFU/ml)	References
S, aureus; S. saprophyticus	Vancomycin	Magnetic nanoparticles were modified with vancomycin to selectively trapped target pathogens.	Immuno-magnetic separation (IMS); MALDI-TOF MS	7 x 10^4	[74]
E. coli 0157:H7	Antibody	Biotinylated polyclonal antibiotics were conjugated with streptavidin-coated magnetic nanoparticles to form a complex.	Immuno-magnetic separation (IMS); Plating	8	[75]
Listeria monocytogenes	Antibody	Magnetic nanoparticles modified with carboxylic acid and antibody.	Immuno-magnetic separation (IMS); Real-time PCR	226/0.5 ml	[76]
E. coli 0157:H7; S. enterica; V. cholera; C. jejuni	Oligonucleotide probe	Streptavidin-coated magnetic nanoparticles hybridized with biotinylated oligonucleotide probe target bacteria cell.	Microscopy (CCD Camera)	316	[81]
S. typhi	Antibody	Magnetic nanoparticles conjugated with antibody was signal amplified upon the addition of horseradish peroxidase.	Chemiluminescence	10	[78]
S. typhi	Monoclonal Antibody (MAb)	Monoclonal antibody coupled with magnetic nanoparticles form complexes with bacteria cell, and enhances trapping on nitrocellulose filter.	Immuno-magnetic capture	2 x10^1	[77]
S. typhi	Aptamer	Development of magnetic nanoparticles modified with aptamers and optical signal amplified with 3',3',5,5'-tetramethylbenzidine (TMB) in the presence H_2O_2.	Colorimetric	NA	[79]
S. entiritidis; E. coli O157:H7; B. cereus	Carbohydrate ligands	Glycan and cysteine-glycan were aggregated on magnetic nanoparticle for microbe extraction.	Magnetic capture	2.19 in *B. cereus*	[82]
S. aureus; A. junii; V. harveyi; M. luteus	Urease	Quarternized magnetic nanoparticles optical signal amplified with urease, urea solution and phenol red.	Colorimetric; Microplate reader @ 558nm	10^2	[72]
E. coli; S. aureus	Chitosan	Positively charged chitosan were coated on magnetic nanoparticles and signal amplified with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in the presence of H_2O_2.	Colorimetric	10^4 (Naked eye 10^2 (Spectroscopy)	[85]

Table 4. Some Bacteria Detected in Functionalized Metal oxides Nanoparticles

NPs used	Target Organisms	Bioreceptor Used	Developed Mechanisms	Detection Methods	Limit of Detection (CFU/ml)	References
SiO_2NPs	*E. coli* 0157:H7	Antibody	Attachment of monoclonal antibodies on surfaces of RuBpy-doped silica nanoparticles.	Microscopy (Fluorescent); Flow cytometry	1 cfu/g	[86]
	E. coli; S. typhi; S. aureus	Antibody	Co-encapsulation of tandem dyes for signal amplification on silica nanoparticles conjugated with monoclonal antibodies.	Microscopy (Confocal)	NA	[87]
TiO_2NPs	*Salmonella*	Antibody	Deposition of monoclonal antibodies coated titanium oxide on glass surface to trap target pathogen.	Photoluminescence	10^3	[90]

3.1.4. Functionalized Bi-Metallic Nanoparticles

The formation of unique nanocomposites with the combination of two metals suggests their potential and importance as composite for the detection of pathogens (Table 5). Duan *et al.* [91] conducted a sandwich-like detection strategy functionalizing Au-Ag nanocomposites with aptamer and X-rhodamine (ROX)-modified aptamer indicating that two noble metals could co-exist as bi-metallic nano-materials for the detection of pathogens.

Interestingly, this was also the case of magnetic and metallic nanoparticles in the formation of nanocomposites [2]. Both magnetic and noble-metal nanoparticles have been studied and extensively applied as nanocomposites. Large nanocomposites of noble-metal and magnetic nanoparticles (larger than 100nm) producing a good magnetic response, improved stability and multiple bio-functionality properties have been established [92,93]. Antibody modified gold coated-magnetic nanoparticles (Ab-Au-MNPs) was extensively studied for the immuno-magnetic separation and colorimetric detection of *E. coli* and *Salmonella choleraesius* [94,95]. Complementing antibody-modified nanocomposites (magnetic and gold nanoparticles) accomplished improved sensitive and selective detection of Group A *Streptococcus pyogenes* [96].

Bi-functional assessment of magnetic nanoparticles using an assembly of polyethylenimine-modified and gold-coated approach showed strong magnetic responsiveness for negatively charged surfaces of bacteria cell [2,45]. Another combine approach describing bi-functional prowess is the novel study which involves the use of vancomycin (antibiotics) SERS tag and aptamer modified Fe_3O_4-Au nanoparticles as dual-recognition tool for the detection of *S. aureus* [97]. The vancomycin-SERS strategy improves signal and sensitive quantification as it

transforms the corresponding aptamer. Wang *et al.* [98] in their study validates the usefulness of vancomycin-modified silver-coated magnetic nanoparticles (Van-Ag-MNPs) and silver-coated gold nanoparticles (Au-Ag) to capture *E. coli*, *S. aureus* 04018 and Methicillin-Resistant *S. aureus* (MRSA) effectively.

Alternatively, the importance of modifying silica-coated magnetic nanocomposites (SiO_2-MNPs) has shown higher capturing efficiency. Ji *et al.* [99] discussed the use of non-noble metal (silica) as the coating shell for MNPs (Fe_3O_4). Amagliani *et al.* [100] established the use of oligonucleotide probe to surface modify SiO_2-MNPs. Other work assessed D-mannose-lectin and 3-Aminopropyltriethoxysilane as the functionalizing bio-elements respectively [70,101]. The method showed a relatively good magnetic response and microscopy detection (with the use of fluorescein-labeled Concanavalin A), identifying the possibility of functionalized/enhanced silicon shell for magnetic nanoparticles. Similarly, Bai *et al.* [102] in their quest for the detection of *Acinetobacter baumannii* focused their novel mechanism on the use of bacteriophages tail-fibre protein -TF2 and TF6, as the functionalizing bio-element for alumina-coated magnetic nanoparticles. This authenticates the peculiarity of tail fibres attachment of bacteriophages to their bacteria host cells.

Table 5. Some Bacteria Detected in Functionalized Bi-metallic Nanoparticles (Nanocomposites)

NPs used	Target Organisms	Bioreceptor Used	Developed Mechanisms	Detection Methods	Limit of Detection (CFU/ml)	References
MNPs & AuNPs	*E. coli*	Polyclonal Antibody	Formation of biotinylated polyclonal antibodies on avidin-modified, gold-coated magnetic nanoparticles traps target pathogen and signal amplified with 5,5-dithiobis-(2-nitrobenzoic acid) coated gold nanorods.	Immuno-magnetic separation (IMS); Surface Enhanced Raman Scattering (SERS) measurement	8	[94]
	E. coli BL21; S. aureus	Polyethylenimine (PEI)	Formation of polyethylenimmine-modified gold-coated magnetic nanoparticles conjugates for effective magnetic capturing response	Magnetic capture; Surface Enhanced Raman Scattering (SERS) measurement	10^3	[2]
	S. aureus	Polyethylenimine (PEI)	Formation of polythylemimine-modified gold-coated magnetic nanoparticles fused with manganese	Immuno-magnetic separation (IMS); SERS measurement	10^9	[45]
	Salmonella choleraesius	Monoclonal Antibody	Monoclonal antibody-modified, gold-coated magnetic nanobeads were immobilized on Immunochromatographic strip pad.	Colorimetric	5	[95]
	S. aureus	Aptamer; Vancomycin SERS tag	Dual recognition method involving the use of Aptamer-modified gold-coated magnetic nanoparticles and Vancomycin SERS tag-coated AuNPs	Microscopy (TEM); SERS measurement	3	[97]
	Group A Streptococcus pyogenes	Antibody	Collection of target pathogen using antibody-modified, gold-coated magnetic nanoparticles and signal amplified with Pyrrolidonyl arylamidase (PYR) and drops of 4-(dimethylamino)-(innamaldelyde (DMACA)	Colorimetric (Visual & Image Analysis); Microscopy (SEM)	3.3×10^2	[96]
MNPs & AgNPs	*E. coli BL21; S. aureus 04018; MRSA*	Vancomycin; Polyethylenimine (PEI)	Development of the combination of polyethylenimine and vancomycin-modified silver-coated magnetic nanoparticles and silver-gold nanoparticle conjugates.	Magnetic separation; SERS measurement	5×10^2	[98]
	Listeria monocytogenes	Oligonucleotide probe	Fusion of oligonucleotide probe of selected pathogen gene with silica-coated magnetic nanoparticles.	Immuno-magnetic separation; PCR	10	[100]
MNPs & SiO_2NPs	*E. coli*	Mannose	Mannose binding lectin was immobilized on silica-coated magnetic nanoparticles and signal amplified with fluorescein-labelled concanavalin A.	Microscopy (Fluorescent)	10^4	[70]
	S. enteritidis; Listeria monocytogenes	3-Aminopropyltriethoxysilane	Magnetic extraction of target bacteria cell DNA using amino-modified silica-coated magnetic nanoparticles complex.	Magnetic extraction; PCR	8 (SE) 13 (LM)	[101]
MNPs & Al	*Acinetobacter baumannii*	Tail fibre protein of bacteriophages (TF2 and TF6)	Formation of TF_2 & TF_6-modified alumina-coated magnetic nanoparticles complexes.	MALDI MS	10^4 & 10^5	[102]
MNPs & PtNPs	*E. coli*	Vancomycin	Attachment of vancomycin to the surface of platinum-coated magnetic nanoparticle (FePt).	Immuno-magnetic separation; Microscopy (SEM; TEM)	180	[103]
Au-Ag	*S. typhi*	Aptamers	Formation of sandwich assay comprising of primary aptamer-modified gold-silver nanocomposite, target pathogen and X-rhodamine-modified secondary aptamer.	SERS measurement	15	[91]

Table 6. Some Bacteria Detected in Functionalized Nano-materials conjugates

NPs used	Target Organisms	Bioreceptor Used	Developed Mechanisms	Detection Methods	Limit of Detection (CFU/ml)	References
AuNPs & Magnetic nanobeads	*Listeria monocytogenes*	Antibodies (Monoclonal & Polyclonal)	Formation of sandwish assay comprising of biotinylated monoclonal antibody immobilized on streptavidin-coated magnetic nanobeads; trapped pathogen and polyclonal antibody-modified gold nanoparticles capped with urease for optical signal amplification.	Colorimetric; UV-Vis Spectroscopy @ 588 nm)	1.0×10^2	[104]
	S. typhi	Antibodies (Monoclonal & Polyclonal)	Development of monoclonal antibody-modified magnetic beads to trap targeted pathogen and polyclonal antibody-modified gold nanoparticles as sandwich complex on carbon electrode.	Immuno-magnetic capture; Electrochemical	7.7×10^1	[105]
AgNPs & Magnetic beads	*S. aureus*	Aptamer	Formation of biotinylated primary aptamer immobilized on streptavidin-coated magnetic beads, trapped pathogen and secondary aptamer-modified silver nanoparticles as sandwich complex.	Immuno-magnetic separation; Electrochemical detection	41	[107]
AuNPs & MMPs	*S. typhi*	Antibody, Aptamer	Aptamer-modified magnetic microparticles from a sandwich with pathogen and detector antibodies-coated on gold nanoparticles and the signal amplified with horseradish peroxidase and reporter antibodies	colorimetric ELAAS, chemiluminescent ELAAS or nano-ELAAS	1×10^3	[106]
MNPs & UCNPs	*S. typhi; S. aureus*	Aptamers	Formation of sandwich assay comprising of primary aptamer-modified magnetic nanoparticles, targeted pathogen and aridin and secondary aptamer-coated up conversion nanoparticles.	Microscopy (Fluorescent); Luminescent signals	5 (ST) 8 (SA)	[108]
	S. aureus; V. parahemolyticus; S. typhimurium	Aptamer	Development of amino oligonucleotide and magnetic nanoparticles together with aptamer-modified oleic acid capped upconversion nanoparticles.	Luminescence	25 (SA), 10 (VP), 15 (ST)	[109]

Platinum-coated magnetic nanoparticles (FePt) conducted on *E. coli* showed stability and solubility in water when modified with vancomycin (Van-FePt) [103]. Although, it is expected for vancomycin to show greater affinity for Gram-positive bacteria, yet, low concentration binding was observed in *E. coli* (Gram-negative bacteria).

On the other hand, surface modification and conjugation of different shape of inorganic metals had received some attention. Sandwich assay complex involving the aggregation of specific monoclonal and polyclonal antibodies on the surface of magnetic nanobeads and AuNPs respectively detect *Listeria monocytogenes* and *S. typhi* cells [104,105]. Wu *et al.* [106] examined the effect of aptamer-modified magnetic microparticles linked with antibody-coated gold nanoparticles. In contrast, a sandwich complex involving two aptamers (primary and secondary) was assessed by Abbaspour *et al.* [107] on magnetic beads and AgNPs. The findings showed the magnetic beads act as the carrier of the affinity ligands, ensuring fast magnetic separation. The two methods advocate their importance to colorimetric, immune-magnetic separation (IMS) and electrochemical detection of their respective target cells.

Huge difference to the previously described nanocomposites was the immobilization of aptamers as molecular recognition elements to surfaces of magnetic nanoparticles-coated upconversion nanoparticles (UCNPs). This was designed to efficiently capture and concentrate *S. aureus*, *S. typhi* and *V. parahaemolyticus* using the magnetic field and in the same vein amplified the luminescent signals [108,109].

Studies reviewed on nanocomposites established the importance and incorporation of iron oxide and magnetic nanoparticles as one of the bi-metallic components. This shows they possess fast and unique capturing properties but, also show a loss in reactivity level, specific surface area, porosity, dissolution rate, aggregation behavior, and stability after certain period. This deficiency is factored by its mineral structure [110,111]. To achieve effective applications of both iron oxide and magnetic nanoparticles, stable reactivity and longer shelf-life in different solutions is quite essential. The physical and chemical stability possessed by other pure and noble metals were observed to improve these defects in iron oxide and magnetic nanoparticles and does not affect their magnetization property when used as nanocomposites as shown in Table 6 [112].

4. Application of Functionalized Inorganic NPs for Pathogens Detection in Food and Water Samples

Food and water safety is an important measure that human and countries prioritize to certify their well-being [113]. The application of the bio-functionalized inorganic nanoparticles had in some ways improved the desire to safeguarding food products against contamination and all form of pathogen infestation. Few of the pathogens closely monitored both in developed and developing countries as the major food and water borne pathogens were practically detected via the application of functionalized inorganic nanoparticles assessed in this review. Seafood safety had received more attention due to the increase in their global demand [114]. Direct infestation, transmission and spread of these contaminated

products by bacteria, biotoxins and other materials had consistently been reported in many countries [115,116]. Shrimps spiked with *S. aureus*, *V. parahemolyticus* and *S. typhimurium* were detected with AuNPs and MNPs-UCNPs functionalized with aptamers [67,109]. The co-existence of the three samples were reported to be efficiently and simultaneously detected in the samples. Wen-de *et al.* [53] achieved the detection of *Streptococcus agalatiae* injected in tilapia using an antibody and AuNPs-laced immunochromatographic strip. The finding reports a detection time of 15 minutes and further assured the validity of the strip for period of 4-6 months. AuNPs conjugated with aptamer application was verified on smoked salmon samples spiked with *Shigella flexneri* gaining a rapid detection time within 20 minutes of interaction [65].

In a similar attempt, attention was also drawn to pathogen detection in dairy products especially milk. Short shelf-life and easy contamination is mostly common in this product. Studies evaluating the detection of pathogens in spiked milk using biofunctionalized inorganic nanoparticles has been reported [68,76,91,95,100,101,106,117]. Investigation by Ma *et al.* [63] on the colorimetric detection of *S. typhi* using aptamer modified-AuNPs had 96.4% recoveries similar to those obtained using the plate counting methods (104.3%). Matta and Alocilja [82] study which spanned for several months examined a total of 18 experiments as it considered the use of carbohydrate-functionalized MNPs. The study put into consideration environmental variations determining the proliferation of *S. enteritidis*, *E. coli* O157:H7 and *B. cereus* in milk samples. The capture efficiency of their analyses were reported to range between 73 to 90 %. Antibodies modified-nanocomposites detect the presence of *S. typhi* in milk spiked with two different concentration of the pathogen [105]. The developed method showed acceptable recovery values range of 91.5% and 106.8%.

Studies on artificial contamination of farm meat such as beef [75,86], pork meat [64,66] and chicken breast [69] were conducted as a case study to test its applicability for real time detection of pathogen in these products as well as similar products. Oh *et al.* [64] reported the usefulness of gold nanoparticle-aptamer-based LSPR sensing chip for *S. typhimurium* detection in pork meat. The study suggests an increase in the recovery rate as the proliferation of pathogen increases in the analyzed sample. The assessment of green products using MNPs have been reported. Chen *et al.* [104] illustrated the detection of *L. monocytogenes* in lettuce plants using antibodies functionalized nanocomposites (AuNPs and magnetic nanobeads). The feasibility of the method was established producing both colorimetric and optical reading at 588 nm. Similarly, Pang *et al.* [97] reported 50 minutes detection time for the detection of *S. aureus* in orange juice as it employs the use of inorganic nanoparticles bio-functionalized with an aptamer and antibiotics. Antibiotics-SERS tag used improves the recovery rate from 95.0% to 106.4%.

The presence of ETEC, *E. coli* BL21 and *S. aureus* were determined with a polyethylenimine-coated inorganic nanoparticles in artificially contaminated water [2,57]. The detection strategy showed rapid magnetic capture, visibility to the naked eye after 2-3 hours of interaction and optical readability within 10 minutes. Work by Huang *et al.* [59] reports gold nanoparticles modified with 4-MPBA produces both colorimetric and photographic detection outcome on 5 isolates (*E. coli*, *S. pullorum*, *S. aureus*, *E. faecalis*, *S. mutans*) within 20 minutes of analysis. Real water samples collected from lake, stream and puddle, without any form of pre-treatment were assessed for real-time detection of *E. coli*, *S. typhi*, *S. aureus* [94,108]. A comparison of the reported biofunctionalized methods with standard plate counting methods showed an average of 95.1% accuracy. Similarly, report by Abbaspour *et al.* [107] from real tap and river samples produced rapid results within 70 minutes of interaction with the aptamer modified inorganic nanocomposites.

5. Advantages of Inorganic Nanoparticles to Signal Amplification

Research findings widely recognized the usefulness of functionalized inorganic nanoparticles to pathogen detection. Several functionalizing strategies described has shown bright potential towards the improvement of biosensor methods for pathogen detection. Functionality gained had increased its use both in conventional and mass spectroscopy method of detection [118]. Conventionally, it improves assessment with the naked eye, absorbance quality and facilitates ionization of bacterial cell surfaces due to their adherence. However, the challenge of cost-effectiveness is still on the high side. Some nanomaterial-based approach still involved the use of expensive reagents and materials which are only available in the laboratory. Despite this challenge, move towards the development and use of simple materials such as paper-based (strip) methods and cotton buds [119] have gained much attention. The approach and efficiency of strip methods to detect several analytes such as uric acid and glucose in body fluids influence the application towards the detection of a pathogen. This boosted the integration of paper-based method as a portable sensing and colorimetric detection device in food and water samples as seen in some of the reviewed studies. A typical example is the fabrication of an immunochromatographic strip, which has aided colorimetric detection mechanism and provides easier detection and assessment with the human naked eye. This technique has shown the possibility of its usefulness as a first-hand indicator in pool samples prior to the comprehensive subjection to complex laboratory screening for food and water samples. A patent technique (NG/PT/NC/2019/3865) established the importance of PLL-functionalized AgNPs as a colorimetric and optical detection tool on different strains of *E. coli* (EPEC, ETEC, EIEC, STEC and EAEC) within 2 hours of interaction. This investigation further adjudged functionalized inorganic nanoparticles-based detection as a promising, prospect-filled approach for rapid pathogen detection, and also informed that there may be little hurdles for its complete achievement, approval and acceptance.

Incorporation and combination of functionalized inorganic nanoparticles with molecular biological tools

such as DNA extraction and PCR reagents as reported in some reviewed studies is gradually enhancing its application in rapid, real-time and pathogen detection [120]. Other works by Li *et al.* [117] and Du *et al.* [121] documented that inorganic nanoparticles could still produce some colorimetric signals without binding to any bio-receptor. This suggests that direct attachment to PCR products with some concentration of salt solution ensures the accuracy of PCR products by eliminating false-positive results.

6. Conclusion

The aggregation of biomolecules on nanoparticles functionalizes and increases their binding affinity to target cells. Furthermore, it shows that the choice of an appropriate bio-recognition element is vital for rapid pathogen detection even at a low level. Therefore, the incorporation of suitable bio-receptors to functionalize these inorganic nanoparticles pave the way to the development of reliable miniature and more affordable tools able to detect food and waterborne pathogens. It is noteworthy that the quest for nanotechnology in the detection of the foodborne pathogens has drastically improved. Nevertheless, there is a still need to improve more on the known strategies which would readily detect both indicator and non-indicator but harmful microorganisms. The food and water industry should embrace, support and provide the needed support that will refine innovative ideas on functionalized nanotechnology inclusion for the detection of pathogens and toxins in food and water products.

Competing Interest

The authors have no competing interests.

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