NANOMEDICINE IN TREATMENT OF TYPHOID FEVER: A REVIEW

Abstract:

For effective management of typhoid, diagnosis of the disease must be done with speed and accuracy. Laboratory diagnosis of typhoid fever requires isolation and identification of Salmonella enterica serotype Typhi. In many areas where the disease is endemic, laboratory capability is limited. Recent advances in molecular immunology have led to the identification of sensitive and specific markers. Currently, alternative methods for biological molecular analysis are enzyme immunoassay, surface plasmon resonance and electrochemical immunoassay. With the development of nanotechnology, various nanoparticles and nano-quantum dots have been used as labels to enhance the sensitivity of the electrochemical immunoassay technique.

Keywords: Typhoid fever, Typhoid diagnosis, Nanotechnology, Nanoparticles.

1. Introduction:

Typhoid fever remains a serious health problem in many regions of the world. The major causes of typhoid fever are caused by Salmonella enterica serovar Typhi (*S. typhi*) and also, to a lesser extent, strains of *S. enterica* belonging to serovars Paratyphi (*S. paratyphi*) A, B and C. This is a highly adapted, human-specific pathogen occurring more frequently in underdeveloped regions of the world where overcrowding and poor sanitation are prevalent. According to the best global estimates, there are at least 16 million new cases of typhoid fever each year, with 6,000,000 deaths. Between 1-5% of patients with acute typhoid infection have been reported to become chronic carriers of the infection; depending on age, sex and treatment regimen. Furthermore, this chronic carrier state has also been implicated in causation of carcinoma of the gall bladder. The diagnosis of typhoid fever on clinical grounds is difficult, as the presenting symptoms are diverse and similar to those observed with other common febrile illness, such as malaria and nonsevere dengue fever. The isolation of serotype Typhi from blood remains the method of choice for the laboratory diagnosis (1).

Classical methods are usually used to detect *S. typhi*, including culturing, serological methods, such as slide agglutination and the Widal test, and polymerase chain reaction (PCR). Even though these methods can provide highly sensitive results for both qualitative and quantitative analysis, they are quite hard- and time-consuming to perform. With the above-mentioned drawbacks, efforts to develop a method for *S. typhi* determination with increased sensitivity and selectivity and a reduction in analysis time needs to be proposed [32,33]. Currently, alternative methods for biological molecular analysis are enzyme immunoassay, surface plasmon resonance, and electrochemical immunoassay. In particular, the use of electrochemical immunoassay has attracted considerable interest for S. 110 typhi determination because of its inherent simplicity, high sensitivity, inexpensive instrumentation, and miniaturization. With the development of nanotechnology, various nanoparticles and nano-quantum dots have been used as labels to enhance the sensitivity of the electrochemical immunoassay technique (2).

Recently, copper, Silver, and gold-enhanced colloidal gold have been reported for immunoglobin G (IgG) determination, which is the model of electrochemical immunoassay with low detection limits ranged from 1.0 ng/mL to 0.25 pg/mL. The metal-enhanced colloidal gold electrochemical stripping metallo-immunoassay combines the high sensitivity of stripping metalanalysis with the remarkable signal amplification resulting from the catalytic precipitation of metals onto the gold nanoparticles (3).

2. Epidemiology

In recent years there have been some changes in the epidemiological patterns of typhoid and related diseases in the third world countries, involving basically most of the countries in Africa, Asia and Latin America.More than 20 million cases a year occur in the hygienically compromised areas of developing countries and out of them Pakistan, India, and Bangladesh together bear the brunt of the attack accounting for 85% of the cases occurring globally.7 Obviously, the highest age-specific rates of typhoid and allied diseases are borne by children and young adults. Studies in Pakistan and Bangladesh show the mean age of patients affected with typhoid fever is 7 years.Typhoid is found to be a seasonal disease; in the monsoon itself there is occurrence of 45% of the total annual reported cases (4).

In South Asia the disease occurrence is highest during July to October because of heavy rainfall during that period.8 Proper standardization of the methods of epidemiological

studies on typhoid is therefore deemed necessary.Buckle et al did an elaborate review using standardized survey methods with 24 studies that examined typhoid fever incidences and employed blood culture as the criteria for diagnosis.We also identified five advanced surveillance reports where incidences of blood culture-confirmed typhoid fever cases were studied.Another very recent published work on the same context was also found.In total, taking all these standardized studies, typhoid epidemiology data were abstracted from 47 countries across the entire global regions. Data were also obtained from population-based and prospective vaccine studies for 13 countries (5).

The remaining incidence data were collected by typhoid fever surveillance systems in the several developed regions where regular and systematic national-level surveillance was in vogue. Paratyphoid fever incidence data were available for only 9 countries of which the USA, despite having an advanced and regular surveillance system, did not have even a single case of paratyphoid fever during the entire period of their study. The incidence of typhoid was high (>100 cases per 100,000 population per year) in Asia (excepting Japan) and Southern Africa (6).

3. Etiology

The main causative agent of typhoid fever is *Salmonella typhi* and *Salmonella paratyphi*, both are members of the Enterobacteriaceae family. *Salmonella* is a genus that has two species *Salmonella enterica serovar* and *enteritidis* classified through extensive analysis by multiplex quantitative polymerase chain reaction (PCR). Both *Salmonella typhi* and *Salmonella paratyphi* (A, B, C) are *Salmonella enterica* serotypes. Nontyphoidal salmonella (NTS) is more typical in children and is mostly limited to gastroenteritis (7).

Salmonella is transmitted by the fecal-oral route through contaminated water, undercooked foods, fomites of infected patients, and is more common in areas with overcrowding, social chaos, and poor sanitation. It is only transmitted from an infected person to another person, as humans are its only host. Major sources of salmonella are poultry, eggs, and rarely turtles. In one study done on the distribution of salmonella isolates by whole-genome sequencing in chicken slaughterhouses in China, 57% of samples were positive (8).

Normal flora of the gut is protective against the infection. The use of antibiotics such as streptomycin destroys the normal flora, which heightens its invasion. Malnutrition decreases

normal gut flora and thus increases the susceptibility to this infection as well. Hence, the use of broad-spectrum antibiotics and poor nutrition amplify the incidence of typhoid fever (9).

4. Bacteriology:

Salmonella enterica serovar typhi is the causative organism for typhoid fever. The bacterium is serologically positive for lipopolysaccharide antigens O9 and O12, protein flagellar antigen Hd, and polysaccharide capsular antigen Vi. The Vi capsular antigen is largely restricted to *S. enterica* serotype typhi, although it is shared by some strains of *S. enterica* sero types Hirschfeldii (paratyphi C) and Dublin, and Citrobacterfreundii.Polysaccharide capsule Vi has a protective effect against the bactericidal action on the serum of infected person (10).

5. Pathogenesis

Between 1000 and 1 million organisms are required to create the disease typhoid in a human being, which therefore is said to be the infectious dose of *S. enterica* serotype typhi. Obviously, *S. typhi* Vi-positive strains are more infectious and more virulent than Vi-negative strains of *S. enterica* serotype typhi. High gastric acidity is one important barrier against invasion of *S. typhi* and a low gastric pH is therefore an important defence mechanism. Aging, gastrectomy, proton-pump inhibitors or antacids leads to achlorhydria and facilitates typhoid infection (11).

In the small intestine, the bacteria first adhere to mucosal cells and then invade the mucosa following which they rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophage that ingest the bacilli but do not generally kill them. Some bacilli remain within the macrophage of the small intestinal lymphoid tissue and some microorganisms translocate to the intestinal lymphoid follicles and the draining mesenteric lymph nodes and by which they enter the thoracic duct and the general circulation (12).

7 to 14 days is usually the incubation period of typhoid. After that there is an interaction between host immunologic mediators and bacterial factors leading ultimately to the necrosis of Peyer's patches. Interestingly, in Africa the disease is often due to nontyphoidal salmonellae such as Typhimurium. In contrast to the Asian situation; however, the two are clinically indistinguishable (13).

6. Symptomatology

Typhoid fever is one of the most common febrile illnesses in developing countries. Following the incubation period of 7 to 14 days, there is onset of fever and malaise. The fever is then accompanied by chills, headache, malaise, anorexia, nausea, vague abdominal discomfort, dry cough and myalgia. These are followed by coated tongue, tender abdomen, hepatomegaly, and splenomegaly. However, recent advances of antibiotic treatment have changed this classic mode of presentation, such as a slow and stepladder type of fever and features of toxicity scarcely seen these days (14).

In areas where malaria is endemic and where Schistosomiasis is common, the presentation of typhoid may be atypical. Even polyarthritis and monoarthritis are reported presentation. Adults often have constipation, but diarrhoea, toxicity and complications such as disseminated intravascular coagulation are more noticeable in infants. Vertical intrauterine transmission from an infected mother may lead to neonatal typhoid, a rare but severe and life-threatening condition. Both relapses and re-infection are common in typhoid and occur in less than 10 per cent of cases. Reinfection can only be distinguished from relapse by molecular typing (15).

7. Diagnosis

The diagnosis of typhoid is usually made in the developing world from clinical criteria. In areas of endemic disease, fever without evident cause that lasts for more than one week should be considered typhoid until proven otherwise. However, malaria, deep abscess, tuberculosis, amoebic liver abscess, encephalitis should also be considered for differential diagnosis (16). Over and above, the following complications of typhoid should be kept in mind as they are often confusing factors during diagnosis and treatment:

- **Abdominal:** Gastrointestinal perforation, gastrointestinal haemorrhage, Hepatitis, Cholecystitis (usually subclinical).

- **Cardiovascular:** Asymptomatic electrocardiographic changes, Myocarditis, Shock.

- **Neuropsychiatric:**Encephalopathy, delirium, psychotic states, cranial or peripheral neuritis, Guillain- barre syndrome, meningitis, impairment of coordination.

- **Respiratory:** Bronchitis Pneumonia (Salmonella enterica serotype typhi, Streptococcus pneumoniae).

- **Hematologic:** Anaemia, Disseminated intravascular coagulation (usually subclinical), thrombocytopenia, haemolytic uremic syndrome.

- **Others:**Focal abscess, pharyngitis, miscarriage, relapse, chronic carrier, influenza, dengue, leptospirosis, infectious mononucleosis, brucellosis, rickettsial diseases etc. should be considered (17).

- **Routine blood tests:** Fifteen to 25% patients show leucopoenia and neutropenia. Leucocytosis found in intestinal perforation and secondary infection.61 In younger children, leucocytosis is common association and may reach 20,000-25,000/mm^3.
- **Liver function tests:** These may be deranged. Although significant hepatic dysfunction is rare, some studies and case reports showed there was hepatic derangement simulating acute viral hepatitis and also present as hepatic abscess (18).
- **Blood culture:** This is the standard diagnostic method; it is positive in 60 to 80 per cent of patients with typhoid. Culture of the bone marrow is more sensitive, around 80 to 95 per cent patients, even in patients taking antibiotic for several days, regardless of the duration of illness. Blood culture is less sensitive than bone marrow because there is lower number of organisms in blood than bone marrow. The sensitivity of blood culture is higher in the first week of illness, increases with the volume of blood cultured (10- 15ml should be taken from school-children and adults, 2- 4ml are required from toddlers and preschool children). Toddlers have higher level of bacteraemia than adult.

- **Other cultures:** Cultures have also been made from the buffy coat of blood, streptokinase treated blood clot, intestinal secretion (with the use of duodenal string capsule), and skin snips of rose spots. The sensitivity of stool culture depends on the amount of faeces cultured, and the positivity rate increased with the duration of illness. Stool cultures are positive in 30 per cent of patients with acute typhoid fever.Urine culture have got 0-58% sensitivity (17).

- **Felix-Widal test:** The classic Widal test is more than 100 years old.It detects agglutinating antibodies to the O and H antigens of *S. enterica* serotype typhi. The levels are measured by using doubling dilutions of sera in large test tube.Although easy to perform, this test has moderate sensitivity and specificity.58 Its reported sensitivity is 70 to 80 per cent with specificity 80 to 95 per cent. It can be negative in up to 30% of culture proven typhoid fever, because of blunted antibody response by prior use of antibiotic. Moreover, patients with typhoid may show no detectable antibody response or have no demonstrable rise in antibody titre. Unfortunately, *S. enterica* serotype typhi shares these antigens with other salmonella serotypes and shares these cross-reacting epitopes with other Enterobacteriaceae. This can lead to false positive results. If paired serums are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic (19).

- **New diagnostic tools:** Tubex test detect IgM antibodies, Typhi dot detect IgM and IgG antibodies against 50 kD antigen of *S. typhi*.Tubex has not been evaluated extensively but in preliminary studies, this test performed better than Widal test in both sensitivity and specificity. Although culture remains gold standard, Typhidot-M is superior to culture method in sensitivity (93%) and has high negative predictive value. In some studies, it has shown that for total Ig estimation ELISA has superior sensitivity when compared to other tests (20).

8. Nanomedicine in Treatment of Typhoid

Nanomedicine is a branch of medicine that applies the knowledge and tools of nanotechnology to the prevention and treatment of disease. Nanomedicine involves the use of

nanoscale materials, such as biocompatible nanoparticles and nanorobots, for diagnosis, delivery, sensing or actuation purposes in a living organism.For more than 2 billion years, microbes have reigned on our planet, evolving or outlasting many obstacles they have encountered (21).

In the 20th century, this trend took a dramatic turn with the introduction of antibiotics and vaccines. Nevertheless, since then, microbes have progressively eroded the effectiveness of previously successful antibiotics by developing resistance, and many infections have eluded conventional vaccine design approaches. Moreover, the emergence of resistant and more virulent strains of bacteria has outpaced the development of new antibiotics over the last few decades. These trends have had major economic and health impacts at all levels of the socioeconomic spectrum – we need breakthrough innovations that could effectively manage microbial infections and deliver solutions that stand the test of time. The application of nanotechnologies to medicine, or nanomedicine, which has already demonstrated its tremendous impact on the pharmaceutical and biotechnology industries, is rapidly becoming a major driving force behind ongoing changes in the antimicrobial field. Here we provide an overview on the current progress of nanomedicine in the management of microbial infection, including diagnosis, antimicrobial therapy, drug delivery, medical devices, and vaccines, as well as perspectives on the opportunities and challenges in antimicrobial nanomedicine(22).

The application of nanotechnologies to medicine, also named nanomedicine, has profoundly altered the landscape of the pharmaceutical and biotechnology industries, with around 100 nanomedicine products already approved for clinical use ranging from drug delivery and imaging to implantable biomaterials and medical devices. Nanotechnologies have also shown impressive potential in tackling almost every aspect of microbial infection (Figure 1). More than 10 nanoparticle-based products have been marketed for bacterial diagnosis, antibiotic delivery, and medical devices (Table 1). With their unique physicochemical characteristics, nanomaterials have played a critical role in the fast, sensitive, and selective detection of microbial infections. Many inorganic and organic nanomaterials have also been demonstrated to possess potent inherent antimicrobial properties that are rarely expressed in their bulk form. More importantly, some of these nanomaterials can combat antibiotic resistance by compromising existing resistance mechanisms. Furthermore, nanoparticles for antimicrobial drug delivery also offer distinct advantages in overcoming resistance and causing fewer side effects than conventional antibiotics. In addition, the incorporation of antimicrobial nanomaterials in medical devices can prevent microbial adhesion and infection. Last, but not

least, using nanomaterials as vaccine adjuvants and/or delivery vehicles can evoke more efficient immune responses against microbial infection. Herein we provide an overview of all these aspects of antimicrobial nanomedicine and discuss the opportunities and challenges of this exciting area(23)

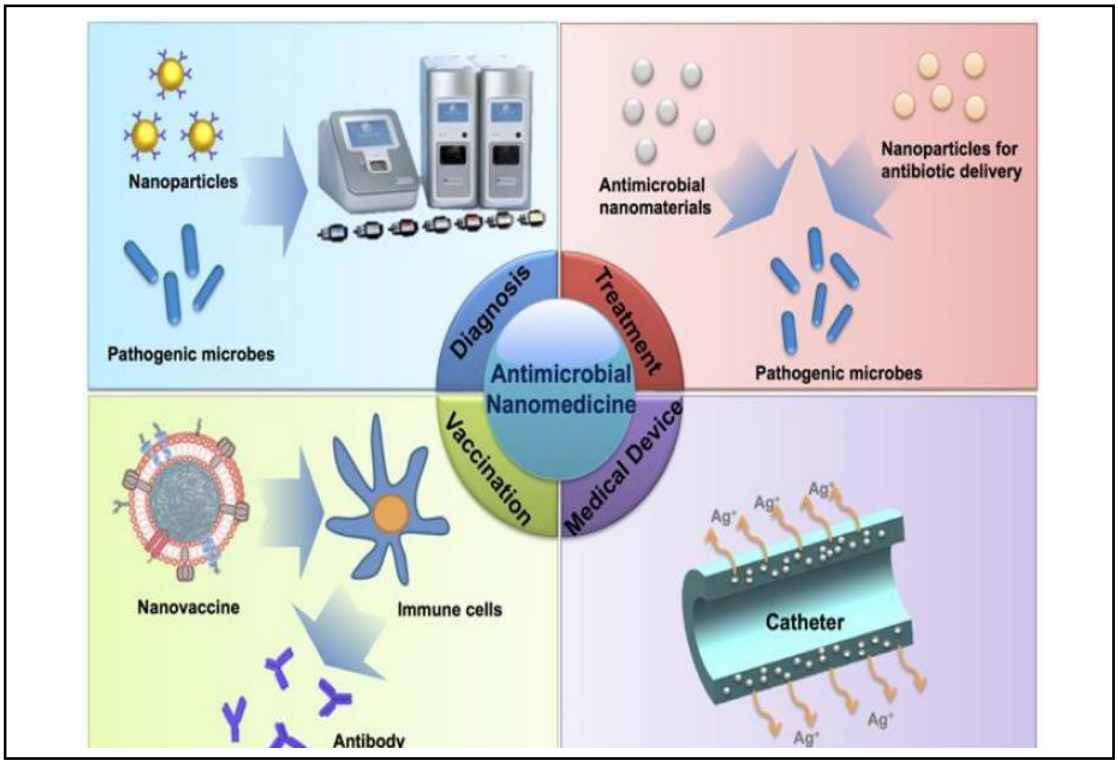

Fig: 1 Nanomedicine applications in the management of microbial infection.

Table 1: Examples of clinical stage nanotechnology-based products for antimicrobial management

	Name	Company/Sponsor	Composition	Application	Stage of Development
Diagnosis	Verigene	Nanosphere	Oligonucleotide-conjugated Au nanoparticle	Bacterial infection and drug resistance diagnosis	Marketed
	T2 Candida	T2 Biosystems	Oligonucleotide-conjugated	Blood detection for sepsis	Clinical trial

			SPION	(candidemia)	
Drug Delivery	Abelcet	Enzon Pharmaceutical	Amphotericin B-lipid complex	Fungal infection	Marketed
	AmBisome	Gilead Sciences	Liposomal Amphotericin B	Fungal infection	Marketed
	Amphotec	Kadmon Pharmaceuticals	Liposomal Amphotericin B	Fungal infection	Marketed
	Fungisome	Lifecare Innovations	Liposomal Amphotericin B	Fungal infection	Marketed
	Arikace	Insmed	Liposomal amikacin	Chronic pseudomonas aeruginosa infection Pulmonary nontuberculous mycobacterial lung disease	Clinical trial (phase 3) Clinical trial (phase 2)
Medical Device	SilvaSorb	AcryMed	Ag nanoparticle-embedded hydrogel	Wound dressing	Marketed
	Acticoat	Smith & Nephew	Nano silver-coated high-density polyethylene mesh	Wound dressing	Marketed
	KoCarbonAg	Bio-medical Carbon Technology	Ag nanoparticle-coated carbon fibre cloth and PE membrane	Wound dressing	Clinical trial
	ON-Q Silver	I-Flow	Ag nanoparticle-coated	Catheter for the delivery of	Marketed

	Soaker		polyvinylchloride	local anaesthetics	
	VentriGuard	Neuromedex	Ag nanoparticle-embedded non-metallic porous materials	Ventricular catheter for cerebrospinal fluid drainage	Marketed
	AGENTO I.C.	C.R. Bard	Ag nanoparticle-distributed hydrophilic polymer	Endotracheal tube	Marketed
	LogiCathAg Tive	Smiths	Ag nanoparticle-embedded polyurethane	Central venous catheter	Marketed
	Silverline	Spiegelberg	Ag nanoparticle- and insoluble silver salt-incorporated polyurethane or silicone	Catheter for internal CSF-drainage	Marketed
	IABN	Hadassah Medical Organization	Quaternary ammonium poly (ethylene imine) nanoparticle-embedded resin	Root canal sealer, and dental restorative materials	Clinical trial (phase 2)
Vaccine	CAF01	Statens Serum Institut	Cationic liposome-based adjuvant	Tuberculosis	Clinical trial (phase 1 completed)

9. Nanotechnologies for Microbial Diagnosis of *Salmonella typhi*

infectious diseases caused by contagious microbes are subject to transmission from either an infected individual or vector to a healthy individual. Rapid, sensitive, and specific detection of pathogens is therefore critical for identifying the source of infection, improving patient

care with proper treatment, and controlling the spread of disease. The complexity and broad variety of microbes, as well as the incubation period before clinical symptoms appear (ranging from a couple of minutes to years after the initial infection), make the diagnosis of some of these conditions very challenging (24).

Modern molecular techniques for microbial infection diagnosis, including enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), possess high sensitivity and reproducibility. Nevertheless, these techniques require laborious sample preparation processes and have long readout times, possibly delaying the time-critical diagnosis of and response to urgent situations in infection, such as bacterial sepsis. Moreover, the sophisticated instrumentation required and short shelf life of some reagents limit the application of these detection techniques in developing countries and rural areas of developed countries, where microbial infectious diseases are more likely a major health problem(25).

Nanotechnology presents a great opportunity for the development of fast, sensitive, specific, and cost-effective techniques for the diagnosis of microbial infection. Sensitive and specific detection requires selective capturing and distinguishing of target molecules/microbes from other substances in a complex sample matrix. Nanotechnology can facilitate both of these processes, and the unique physicochemical properties of nanomaterials potentially enable the recording a single binding event. Targets can be labelled or captured by nanoparticles coupled with affinity probes (e.g., antibodies and nucleic acids), which can selectively recognize microbe biomarkers. In addition, the development of surface patterning techniques and nanoscale arrays of pathogen-targeting ligands may further revolutionize pathogen detection. Several different types of nanomaterials have been used for microbial diagnosis, including magnetic, gold (Au), and fluorescent nanoparticles(26).

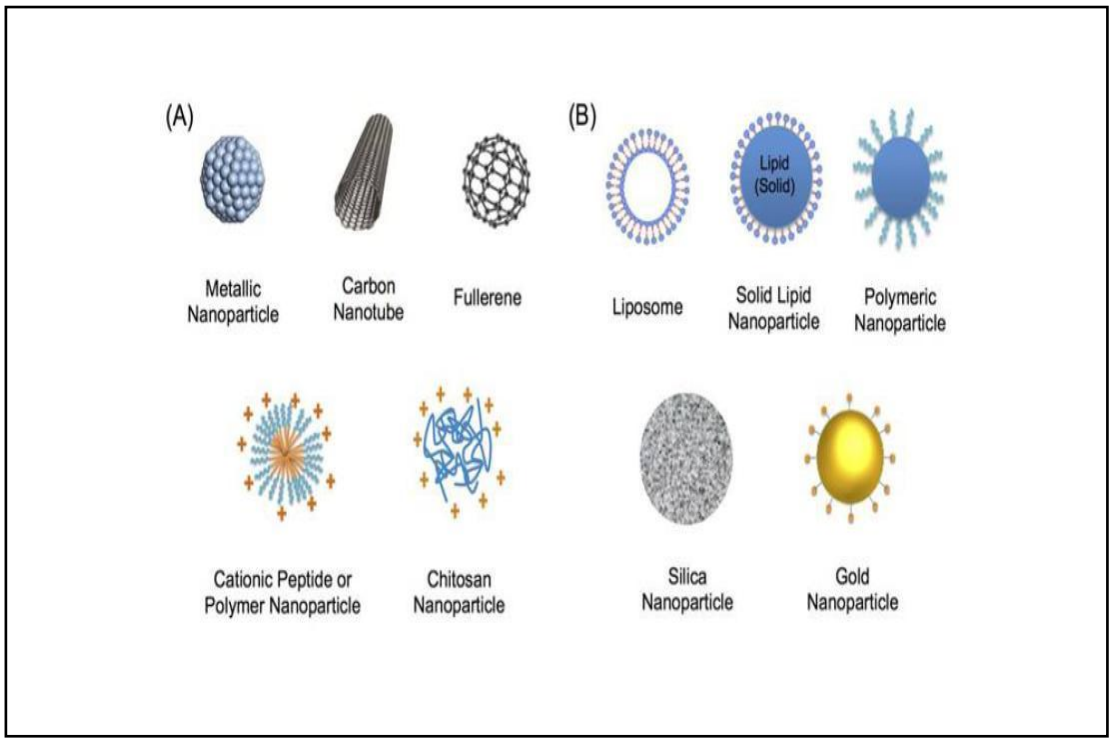

Fig: 2 Schematic of (A) nanomaterials with inherent antimicrobial properties, and (B) nanoparticle-based antimicrobial drug delivery systems.

9.1. Magnetic Nanoparticles

Magnetic nanoparticles, in particular superparamagnetic iron oxide nanoparticles (SPIONs), have been well studied as magnetic resonance imaging contrast agents for medical applications. A great deal of effort has also been focused on the application of probe-decorated magnetic nanoparticles for microbial diagnosis. In one recent study, Ravan and Yazdanparast reportedloop-mediated amplification (LAMP–ELISA) assay for the detection of enteric fever in spiked samples. The spiked samples were incubated up to 24 hours. The hybridization of probe and amplification were performed simultaneously. The spiked samples were detected in 2 hours 40 minutes by measuring absorbance at 450 nm using microplate ELISA reader. The LOD of this assay was reported as 10 CFU/mL. Bozorgmehr et al. used LAMP based non-crosslinking gold nanoprobes for the detection of *S. typhi* DNA. The team used surface plasmon resonance (SPR) for end point-detection. However, in the two methods described above, no clinical blood samples were reported. Abdullah et al. developed an in-house LAMP assay for the detection of *S. typhi* using three sets of primers designed for PapD gene. LAMP reaction was performed using heating block (at 63°C for 60 minutes) followed

by detection using colorimetry. The LAMP method was compared against the gold standard of culture method and polymerase chain reaction (PCR). The team reported a LOD of 10^4 CFU/mL or 20 CFU/reaction while that of PCR was 200 CFU/reaction (27).

9.2 silver Nanoparticles

Specifically, silver NPs have been reported to have microbial inhibition against *Salmonella* strains (*Salmonella typhi* and *Salmonella paratyphi*). Also, a green synthesis of silver NPs has been demonstrated to have antibacterial activity against *Salmonella typhimurium*. It has been further demonstrated that *Salmonella* growth is greatly inhibited by a synergistic antibacterial activity achieved by combining silver NPs with antibiotics. This study is an attempt to investigate how gold nanoparticles (GNPs) affect these bacteria. Nevertheless, it has been reported that the resistance of various enteric human pathogenic bacteria against many synthetic drugs is being enhanced day by day. According to the earlier published results of the antimicrobial activity of GNPs against human bacterial pathogens, it is outlined that there is no significant reaction for the zone of inhibition for *E. coli* and *S. aureus* and only 7 mm and 16 mm were obtained, respectively (28).

9.3 Fluorescent Nanoparticles

Nanomaterials with fluorescent properties or nanoparticles labelled/encapsulated with fluorescent dyes have also been applied for microbial detection. Using antibody-conjugated silica nanoparticles that encapsulate thousands of fluorescent dye molecules for signal amplification, Tan and co-workers developed an assay tool for in situ detection of single bacterium cells in less than 20 min. They also developed multi-coloured FRET (fluorescence resonance energy transfer) silica nanoparticles by co-encapsulating three tandem dyes that emit unique colours upon excitation with a single wavelength. Simultaneous detection of multiple bacterial targets was achieved with different monoclonal antibody-conjugated FRET silica nanoparticles. Quantum dots (QDs), a type of fluorescent semiconductor nanoparticle, exhibit many characteristics that make them superior to conventional fluorophores, such as photobleaching resistance and size-tunable broad absorption spectra with narrow emission (29).

Jain s. and colleges (2016) present work demonstrates effective utilization of functionalized polymeric fluorescent nanoparticles as biosensing probe for the detection of *Salmonella typhi* bacteria on modified polycarbonate (PC) filters in about 3 hrs. Antibody modified-PC

membranes were incubated with contaminated bacterial water for selective capturing which were detected by synthesized novel bioconjugate probe. Core–shell architecture of polymeric nanoparticles endows them with aqueous stabilization and keto-enolic functionalities making them usable for covalently linking *S. typhi* antibodies without any crosslinker or activator. Bradford analysis revealed that one nanoparticle has an average of 3.51 × 10−19 g or 21 × 104 bound *S. typhi* Ab molecules. Analysis of the regions of interest (ROI) in fluorescent micrographs of modified fluoroimmunoassay showed higher detection sensitivity of 5 × 102 cells/mL due to signal amplification unlike conventional naked dye FITC-Ab conjugate. Fluorescence of pyrene dye remained same on immobilization of biomolecules and nanoparticles showed stable fluorescent intensity under prolong exposure to laser owing to protective polymeric layer allowing accurate identification of bacteria. Surface-functionalized PC matrix and fluorescent label NPs permit covalent interactions among biomolecules enhancing signal acquisitions showing higher detection efficiency as compared to conventional microtiter plate-based system. Our novel immunoassay has the potential to be explored as rapid detection method for identifying *S. typhi* contaminations in water (30-31).

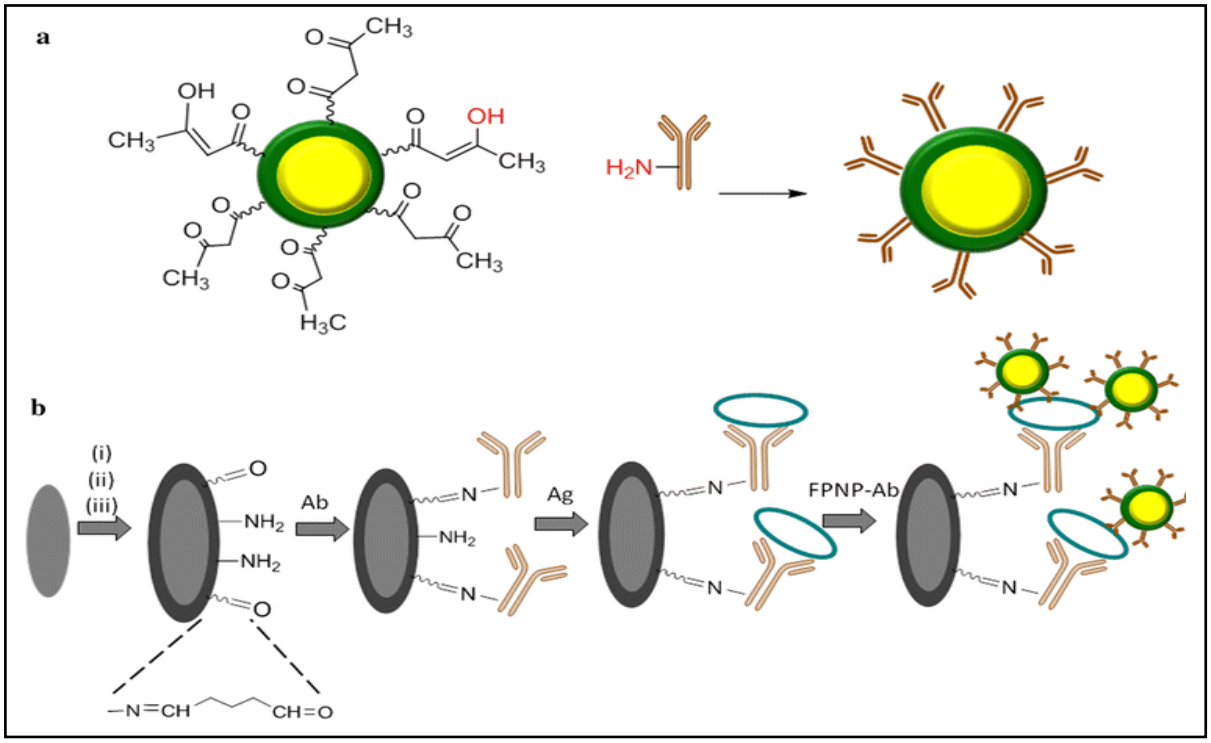

Fig: 3 (a)Covalent linkage between FPNP and *S. typhi*Ab forming bioconjugate probe and (b) Generation of immuno-complex for the formation of mPC fluoroimmunoassay involving addition of (i) nitric acid (ii) sodium borohydride (iii) glutaraldehyde, antibody (Ab), antigen (Ag), and bioconjugate probe (FPNP-Ab)

9. Conclusions:

Nanotechnology is an emerging field that is potentially changing the way we treat and diagnose diseases. The metal-enhanced colloidal gold has not been previously applied to the detection of bacterial cells in real samples, especially for the detection of *S. typhi*. Therefore, one can employ the electrochemical stripping-metallo-immunoassay based on a copper, silver or gold enhanced – colloidal gold nanoparticle label for the determination of *S. typhi* in real samples, which will be useful in the diagnosis, follow-up treatment, and controlling in advance the epidemic disease of typhoid fever. The coupling of gold nanoparticles with the advantages of electrochemical stripping analysis can easily be extended for detecting other bacterial cells in real samples with high accuracy and sensitivity. As someone has truly predicted, there has been plenty of room at the bottom to modify and enhance existing technologies by controlling material properties at the nanoscale. Therefore, with sufficient time and research, the promise of nanotechnology-based disease diagnosis may become a reality.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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