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IRON OXIDE NANOPARTICLES: AN ALTERNATIVE AGAINST DRUG-RESISTANT UROPATHOGENS

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ABSTRACT

Antimicrobial resistance is a major concern worldwide, its misuse led to the development of drug-resistant bacteria and it is one of the greatest challenges for medical practitioners. Also, it has become a significant global threat due to its major cause of morbidity and mortality. Urinary tract infections caused by uropathogens (UPEC, *P. aeruginosa*, *K. pneumoniae*, *Enterococcus faecalis*, *P. mirabilis*) have shown drug-resistance against a wide range of conventional antibiotics and has posed a danger to the human health. The replacement of conventional antimicrobials by nanotechnology developing iron oxide nanoparticles (IONPs) to counteract drug-resistant uropathogens is ongoing. The effectiveness of IONPs depends upon how the pathogens and IONPs interact with each other. The development of effective IONPs requires in-depth knowledge of its physicochemical properties and the biological aspects of urinary tract pathogens. It has been proved that IONPs possess remarkable antimicrobial potential because of its various unique properties, it also has excellent biocompatibility and biodegradability and can be used as nanocarriers during drug delivery. Hence, it is an alternative therapy against drug-resistant uropathogens. The present review discusses about IONPs and its properties, pathogenicity of drug-resistant uropathogens and its interaction with IONPs, its antibacterial effect, biocompatibility, biodegradability, drug delivery systems and future prospects.

KEYWORDS: UTI, drug resistance, uropathogens, iron oxide nanoparticles, antimicrobial mechanism, drug delivery, alternative therapy.

1. INTRODUCTION

Antibiotic resistance has become a major concern all over the world. This crises has developed due to the overuse or misuse of antibiotics.[1] A most serious threat to humanity is the emergence of microbes resistant to antibiotics. There is a rapid emergence of drug resistant bacteria occurring worldwide due to which the efficacy of antibiotics has endangered.[2] The increasing frequency of antibiotic-resistant bacterial infections poses an alarming confusion to health care today. One of the greatest challenges to global health is infectious diseases, it is a major health problem to the developing countries.[3] Urinary tract infections (UTIs) are most common bacterial infections, affecting more than 150 million of people each year globally.[4] Both men and women may be infected but UTIs are more commonly found in women as compared to men. Across their lifespan approximately 50% of women are affected by UTIs.[5,6]

UTIs are classified as uncomplicated or complicated. Uncomplicated UTIs typically affect healthy individuals who have no structural or neurological urinary tract abnormalities; these infections are further differentiated into lower UTIs/cystitis (infection of the bladder) and upper UTIs/pyelonephritis (infection of the kidney). Various risk factors are associated with cystitis, including female gender, a prior UTI, vaginal infection, sexual activity, obesity, diabetes and genetic susceptibility.[7,8] Complicated UTIs occurs in urinary tract that has anatomical or functional abnormalities. These UTIs are associated with predisposing factors that compromise the urinary tract or host defence, including urinary obstruction, urinary retention caused by neurological disease, immuno-suppression, pregnancy, renal failure, renal transplantation and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices. Catheter-associated UTIs (CAUTIs) are affiliated with increased morbidity and mortality. Risk factors for developing a CAUTI include prolonged catheterization, female gender, older age and diabetes.[9-11]

Clinically, bacterial infections of the urinary tract have variety of signs and symptoms and may be caused by range of organisms. The etiologies include UTIs from uropathogenic *Escherichia coli* (UPEC) which is responsible for community acquired infections and *Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Proteus mirabilis, Enterococcus faecalis, Enterobacter*; these uropathogens become particularly pertinent during catheter-associated urinary tract infections (CAUTIs) and hospital-acquired infections.[12] Most commonly treatment of antibiotics is prescribed to the patient suffering

from a symptomatic UTI. Prolonged treatment of antibiotics can alter the normal microflora of the vagina and gastrointestinal tract which will further lead to the development of multidrug-resistant micro-organisms. The availability of niches that are not any longer filled by the altered microbiota can increase the danger of colonization with multidrug-resistant uropathogens.

The development of antimicrobial resistance (AMR) may be caused by alteration or inactivation of the drug or by reduction of binding capacity of the drug due to altered binding sites; reduced antimicrobial effect due to modification of the metabolic pathways; or decreasing permeability and increasing active flux which leads to reduced intracellular accumulation of antimicrobial agents. Although antimicrobial resistance may be intrinsic or acquired; it can develop through horizontal gene transfer or by vertical evolution.[13]

As AMR is a global health and development threat, there is a need for alternative prevention and treatment options for UTIs because of rise in disease incidence globally due to the increased resistance by uropathogenic bacteria to currently used antibiotics. Current therapeutics are suboptimal, as the prevalence of multidrug-resistant uropathogens is increasing and antibiotic treatment for acute infection does not preclude recurrences.[12] Some approved drugs are limited in use due their adverse side-effects.[14] To overcome these crisis researchers have discovered many alternative strategies for prevention and treatment of UTIs using nanotechnology. Golden era of antibiotics is waning, therefore there is an increase in the need for rationally designed and alternative treatments. Various efforts based on metal oxide nanoparticles (NPs) have been made to detect and kill uropathogenic bacteria.[15,16] Over last few years, Iron oxide nanoparticles (IONPs) have extensively been studied and widely used as treatment for UTIs against drug-resistant uropathogens.[17-19] For example, Iron oxide nanoparticles (IONPs) could be functionalized with target molecules such as various antibiotics, antibodies, antimicrobial peptides, bacteriophages and aptamers for bacterial separation and concentration. Furthermore, IONPs have unique magnetic properties and high specific surface area have shown great promise in antibacterial and biomedical applications.[20]

Based on the above facts, the present review is mainly focused on IONPs and its properties, pathogenicity, antibiotic resistant mechanisms of drug-resistant uropathogens, antibacterial activity of IONPs against drug-resistant uropathogenic bacteria and their potential

advantages, drawbacks and future prospects. In addition, this comprehensive information may be useful to study IONPs used against drug-resistant uropathogens.

2. Iron oxide nanoparticles (IONPs)

Iron oxide nanoparticles are most widely used nanoparticles in various fields. It is one of the traditional metal oxides nanoparticles bearing magnetic properties. These nanoparticles are classified according to size in terms of diameter, they cover a range between 1 to 2000 nanometers.[21] Their properties are mostly dependent on the size of the particle. Smaller size of the nanoparticle drives different physicochemical properties like colloidal properties, magnetic properties and optical or electrical properties.[22] IONPs also have unique properties such as superparamagnetism, high surface-to-volume ratio, greater surface area and easy separation methodology. Iron oxide nanoparticles are produced in magnetite, maghemite and hematite forms by physical, chemical and biological methods and it comes under magnetic nanoparticles. Due to the metallic core oxidation iron nanoparticles forms in core-shell form. There are several phases in iron oxide formation and colour differs for each form. For example, magnetite (Fe_3O_4) has a black colour, maghemite (γ-Fe_2O_3) has light brown colours, and hematite (α-Fe_2O_3) has red colours.[23,21] When these IONPs reach smaller sizes for about 10–20 nm, superparamagnetic properties become evident, so that the particles reach a better performance for most of the leading applications. Due to their superparamagnetic properties, IONPs are good contrast agents for in vivo bacterial imaging, also enhances antimicrobial efficacy, protects antimicrobial agents from enzyme deactivation and releases antimicrobial agents in a sustained and controlled manner, improving bioavailability and reducing systemic side effects.

The various synthesis methods have been developed which can be classified as physical methods (a top-down approach, starting from bulk metal that undergoes fractionation into tiny pieces by mechanical action into consecutively smaller fragments) e.g., Aerosol, Gas phase, Electron deposition beam lithography, Power ball milling, Laser-induced pyrolysis, Pulsed laser ablation, etc. Chemical methods (bottom-up approaches are used in chemical methods involving organic solvents) e.g., Thermal decomposition, Co-precipitation, Sonochemical, Hydrothermal, Microemulsion, Electrochemical decomposition.[20] High-quality IONPs are synthesized in organic solvents combined with thermo decomposition of organo-metallic precursors route at high temperatures, which will lead to highly crystalline and monodisperse nanoparticles. More recently developed biological methods which are

focussed on green-synthesis processes use different types of microorganisms. The biological methods such as plant mediated, protein mediated are also used for synthesis of IONPs.[24-26]

Synthesized IONPs can be further characterized by various techniques such as X-ray diffraction, Dynamic light scattering (DLS), Zeta potential (analyses surface charge of nanoparticles), Absorption spectrophotometry, Fourier Transform Infrared Spectroscopy (FTIR), Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM). The microscopic methods are used to generate images of individual nanoparticles for characterization of their shape, size, and location. And spectroscopic methods are used to measure the particles interaction with electromagnetic radiation as a function of wavelength, as it is useful for some classes of IONPs to characterize concentration, size, and shape.[26-28] However, reproducible synthesis of IONPs with desired properties is still a concern. This is due to existing synthesis methods which shows a passive approach towards synthesis reaction and unreacted components effect the final product during undesired reactions, partial mixing of reagents. All these factors make it difficult to achieve reproducibility in the desired properties. To overcome such challenges Immediate post reaction purification of nanoparticles is necessary to minimize error. Direct active and complete mixing strategies for reactants and automated approaches could also solve the issue.[29]

IONPs has been taken special attention by researchers from various fields due to their outstanding multifunctional properties such as small size, high surface area to volume ratio, thermal stability, stability in physiological environments, and size-dependent magnetic properties, high coercivity, high saturation magnetization and low toxicity compared to other nanoparticles. This opens up a new window for medicine, biosensors, and drug delivery fields. Therefore, IONPs are frequently assessed for their potential nanotechnology related applications in magnetic fluids, magnetic recording devices, high-frequency switch modes, electromagnetic absorbers, catalysis, dye/antibiotic degradation, nano-biomedicines, cosmetics, diagnostics (magnetic hyperthermia agents to diagnose and treat various bacterial infections), target drug delivery, nanocarrier for vaccination, biosensing, heavy metal absorption, waste water treatment, material sciences, etc. They are the only type of magnetic nanoparticles that are approved by food and drug administration (FDA) for clinical use.[20,16,30]

Excess irons are processed and stored by human body. After internalization by cells, and hydrolytic enzymes metabolize IONPs (contained in endosomes and lysosomes) into

elemental iron and oxygen, where the iron joins normal body stores. Many efforts have been made to use IONPs for multi-functional diagnosis and therapy purposes.[31] Application of IONPs in composites form with antimicrobial agents showed activities because of their magnetic, biocompatibility, and biodegradability properties. Magnetic property of Fe_3O_4 NPs was used for targeting drug resistant bacteria including uropathogens.[32] Therefore, IONPs are considered to be the most promising tools used and thus various applications in interdisciplinary fields.

3. Pathogenicity and mechanisms of drug-resistant uropathogens

It is well known that micro-organisms have developed resistance to various antibiotics. Evolution of these micro-organisms resulted in the abilities such as drug resistance. However, other mechanisms with biochemical and molecular aspects are involved in acquired resistance of drug resistant uropathogens. For a better understanding, mechanisms of drug resistant urinary tract pathogens are presented in the following sections. Various mechanisms concerning antibiotic resistance which involves target protein mutation, low susceptibility of target protein by acquisition of related genes, antibiotic inactivation by specific enzymes, the target bypassing and application of efflux pump for inhibition of drug access to target.[15]

- **Uropathogenic Bacteria**

Uropathogenic bacteria are pathogens with specific virulence factors that promotes their invasion of the urinary tract. The pathogenesis and mechanisms of some drug-resistant uropathogens are explained in the following subsections.

a. Uropathogenic *Escherichia coli* (UPEC)

Uropathogenic *Escherichia coli* (UPEC) Gram-negative bacteria colonizes the urinary tract, injects in the urethra and ascend into the bladder. UTIs are allied with *E. coli* virulent strains such as O157:H7 and O104:H4. UTIs occur when UPEC is introduced in urinary tract, they adhere and invade into uroepithelial cells in a FimH-dependent manner.[33] By using type 1 pili tipped with the FimH adhesin, they bind specifically to mannosylated receptors on the luminal surface which is present on the mammalian bladder epithelial cells. From the epithelial cells UPEC can be expelled by bladder cells as a part of an innate defence mechanism. Inside the epithelial cell UPEC undergo clonal expansion to form an intracellular bacterial community (IBC). Bacteria get dispersed from the IBC and spread to other cells forming further IBCs and begin new infection cycle.[34,35]

UPEC is the causative organism of more than 85% of all UTIs have become incrementally resistant to antimicrobials. Drug resistant *E. coli* containing plasmids such as CTX-M of antibiotic resistance synthesizes Extended spectrum β-lactamases (ESBLs). The resistance against monobactams, cephalosporins, quinolones, and aminoglycosides was developed by these strains.[36] Also, another resistance mechanism is modification of porins (OmpF, OmpC, and PhoE) in outer membrane of *E. coli*. Here, the expression changes in the type of porins due to lack of porin function. *E. coli* uses efflux pump to expel fluoroquinolones, chloramphenicol, fusidic acid, tetracyclines, β-lactams, and novobiocin by AcrAB-TolC system which is another approach of resistance.[37,15]

b. Pseudomonas aeruginosa

Pseudomonas aeruginosa is one of the notorious Gram-negative pathogens which cause UTIs and CAUTIs. They form biofilms on indwelling medical devices. The virulence factors secreted by *P. aeruginosa* are LecA and LecB, siderophores, pyoverdine, pyocyanin, pili, alginate, hemolysins and exopolysaccharides. These virulence factors play important roles in the pathogenesis of *P. aeruginosa* and its survival, also by colonizing, invading within the host tissues. *P. aeruginosa* has major virulence factors which include proteases (LasA, LasB, AprA, PrpL), exotoxins (A, S, T, U and Y), phospholipase-C, pyocyanin, siderophores and alginate. β-lactamases, efflux pumps and down regulates expression of OprD also produced by *P. aeruginosa* which contributes to antibiotic resistance.

Also, there is development in antibiotic resistance due to genetic mutations in gyrA and parC gene.[38] Other important mechanisms of antibiotic resistance in this bacterium are target-binding site alterations, reduction of internal antibiotic concentrations by lack of porins and application of efflux pumps.[15] *P. aeruginosa* is resistant to drugs by using various mechanisms. Verona Integron-encoded Metallo β-lactamase (VIM) enzyme destructs β-lactams antibiotics such as carbapenemase. The expression of chromosomal or plasmid-mediated AmpC genes resulted in resistance to cephalosporins and β-lactams antibiotics.[37]

c. *Proteus mirabilis*

Proteus mirabilis is ubiquitous and Gram-negative in nature, most common causative agent of CAUTI in long term catheterized patients with low immunity. The enzyme urease is produced by *Proteus spp*. Production of urease hydrolyzes urea to ammonia (NH_3) and CO_2. There is a rise in pH of the urine, facilitating the kidney stone formation. Incorporation of calcium crystals and magnesium ammonium phosphate precipitates into polysaccharide

microbial capsules, further forms crystalline biofilms on the catheter. Formation of crystalline biofilms provides protective niche during antibiotic treatment, which will promote the recurrence.[39]

Proteus mirabilis possesses various virulence factors to establish infection in the urinary tract. These virulence factors include adhesive mannose- resistant *Proteus*- like (MR/P) fimbriae, non-agglutinant fimbriae (NAF), *P. mirabilis* fimbriae (PMF) for adhering to urinary epithelium cells, flagella, antigenic variation, capsule, IgA protease, LPS, and metabolic enzymes such as protease and urease, hydroxyapatite crystal formation, and iron acquisition systems.[40] After the initial attachment of *P. mirabilis*, multiplication of the bacteria and formation of biofilms takes place which protect the uropathogen from the host immune response and antibiotics. Proteus toxic agglutinin (Pta) and hemolysin (HpmA) are the two toxins produced by *P. mirabilis*. Toxin HpmA destabilizes the host cell by inserting itself into the cell membrane and Pta enters into the host cell membrane by producing holes to it which further causes cytosol leakage, osmotic stress and actin filaments depolymerization and also induces cell-to-cell interaction.[41,33] The pathogenesis of *P. mirabilis* is more or less same as UPEC. *P. mirabilis* invades the bladder epithelial cells and then there is formation of IBCs in the urothelium. In a mouse model of CAUTI, co-infection with other uropathogens like *E. coli*, *Enterococcus*, *Klebsiella* or *Pseudomonas* species boosts the urease enzyme activity of *P. mirabilis*, which will result in the increased disease severity and spreading to other organ systems.[42] *P. mirabilis* carries ESBL genes as well as plasmid mediated genes resistance to fourth-generation cephalosporins, macrolides and aminoglycosides.[43]

d. Klebsiella pneumoniae

Klebsiella pneumoniae is a Gram-negative bacterium, medically most important species of the genus, causes nosocomial infections such as UTIs. For them most common site of infection is the urinary tract. The pathogenicity factors of *K. pneumoniae* includes capsule which protects the bacterium from undergoing phagocytosis by polymorpho nuclear granulocytes and by bactericidal serum factors killing of the bacteria is prevented. The bacterial adhesins are Type 1 pili which mannose-sensitive hemagglutinins (MSHA) that agglutinates erythrocytes and Type 3 pili is mannose-resistant/*Klebsiella*-like hemagglutination (MR/K-HA) which helps the bacterium from binding to mucus and adhere to uroepithelial cells. Only *Klebsiella* can synthesize this fimbrial type.[44] Further, colonization progresses to infection.

Lipopolysaccharide (LPS), also termed endotoxin, comprises of lipid A and the O-antigen. Variations in LPS protects bacteria from antimicrobial peptides and polymyxin antibiotics.[45] Extensive spread of drug resistant strains is due to the production of extended-spectrum β-lactamase (ESBL) by *K. pneumoniae*. The presence of CTX-M which is a plasmid-encoded ESBL makes pathogen resistant to cephalosporin. Another resistance mechanism is protection of the binding site of DNA topoisomerase IV and gyrase against quinolone antibiotic. Extended resistance to β-lactam antibiotics has been observed in *K. pneumoniae* due to change in porin proteins from OmpK35 to OmpK36. Another way of antibiotic resistance is expression of blaNDM gene which is plasmid-mediated destructs β-lactams.[37,15]

e. *Enterococcus faecalis*

Enterococcus faecalis is an opportunistic Gram-positive uropathogen causing CAUTIs. When occurs, they cause almost untreatable infections. Resistance was found against vancomycin by more than 7% of *E. faecalis* clinical CAUTI isolates.[46] The fibrinogen- binding adhesin (EbpA) is tipped with Ebp pili that binds to the fibrinogen coating the catheter. The pathogen uses fibrinogen (as a nutrient source) for their growth in urine. The virulence factors GelE and SprE which are proteases secreted by *Enterococcus faecalis*. During infection, the upregulation of these enzymes cleaves the α-, β- and γ- which are chains of fibrinogen to potentiate formation of the biofilm. Utilization of fibrinogen by the *E. faecalis* serves as binding platform and food source during growth and biofilm formation. Host proteases activates SprE that promotes spreading of the bacteria in the kidneys.[47]

E. faecalis showed six PBP genes which are generally considered, three class A genes- ponA, pbpF, pbpZ and three class B - pbp5, pbpA, pbpB. Due to the presence of a species-specific chromosomal gene, pbp5 that encodes a class B PBP which has low binding affinity for ampicillin and cephalosporins, also have tolerance to the action of β-lactams. Inactivation of the antibiotics is mediated by a β-lactamase that cleaves the β-lactam ring; this makes the bacteria resistance to ampicillin. The resistance is mediated by multiple genes. It has been found that in *E. faecalis* clinical isolates the gene cfr encodes methylase (Cfr) which is a plasmid-borne determinant of resistance. Also, tetK and tetL encodes efflux pumps which are plasmid-borne determinants that encodes proteins with 14 α-helices which builds the transmembrane domains and confer tetracycline resistance. Mutations in rpoB gene that encodes for the β-subunit of the RNA polymerase giving rise to rifampicin resistance. rpoB (H486Y) mutation in *E. faecalis* increases resistance to broad-spectrum cephalosporins.[48]

f. Staphylococcus aureus

Staphylococcus aureus Gram-positive bacteria is an uncommon cause of UTIs in the general population but it is a common cause of UTI in patients with urinary tract catheterization. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is the majority isolates found during CAUTIs. For adherence to the fibrinogen- coated catheter *S.aureus* use clumping factor B (ClfB) and enhance inflammation of the bladder to develop persistence. For binding to fibrinogen-coated catheters MRSA strains use microbial surface components recognizing adhesive matrix molecules, they do not encode pili. ClfB plays an active role in MRSA CAUTI pathogenesis, whereas clumping factor A (ClfA) is needed for systemic infection.[33] Catheter-mediated inflammation is further potentiated by MRSA upregulation of the expression of IL-1α, IL-1β, IL-6 and TNF which promotes fibrinogen release and bacterial tenacity in the bladder in a mouse model of CAUTI.[49]

Genetically, the presence of mecA and mecC resistance genes in the MRSA genome and based on this there are two main types of MRSA strain. These resistance genes encode PBP-2a and have a low affinity for β-lactam antibiotics such as penicillin and cephalosporin. Also, β-lactamase enzyme is produced by antibiotic resistance *S. aureus*. β-lactamase enzyme hydrolyze the ability of β-lactam ring. This enzyme is under expression of the activation of blaZ gene.[15] Thus, most β-lactams are inactive against MRSA, the exceptions being ceftobiprole and ceftaroline.[34]

4. Mechanisms of Iron oxide nanoparticles interaction with bacteria

Initially, for better understanding of the IONPs interaction with bacteria, it is important to identify morphological features in IONPs and bacteria. According to cell wall morphology, the bacteria are divided into two main types Gram-positive and Gram-negative, which is used to evaluate antibiotics. Gram-positive bacteria cell wall (20–80 nm) contains thick peptidoglycan layer and cell membrane. Gram-negative bacteria cell wall (10–30 nm) components are the plasma membrane, outer membrane, and thin peptidoglycan.[50] As compared to Gram-negative bacterial cell wall, Gram-positive cell wall is more complex both structurally and chemically. Gram-negative bacteria contain the outer membrane LPS increases the net negative charge of the cell membrane which makes the cell wall impermeable to lipophilic solutes and also limit the penetration of negatively charged free radicals. Thus, Gram-negative bacteria are less sensitive to IONPs. In contrast, Gram-positive bacteria have only the peptidoglycan layer and are more sensitive to lower concentrations of

IONPs due to the less permeability barrier. Therefore, Gram-positive bacteria are more sensitive to IONPs than Gram-negative bacteria and the sensitivity is due to the properties of the cell wall and cell membrane polarity. The effectiveness of Fe_2O_3 nanoparticles is higher against Gram-positive bacteria because of the smaller negative charge of *S. aureus* cell membrane than that of *E. coli* causing a high level of penetration of the negatively charged IONPs. Bacterial cell has adhesion factors which mediates the attachment to various substrates and also mediate the interactions of a bacterial surface with IONPs.[51,52] Based on the properties of the charge, bacterial response to antibacterial agents such as IONPs can be determined. The surface of both Gram-positive and Gram-negative bacterial cell wall is negatively charged, this is due to the anionic properties of teichoic acids and LPS in their cell surface.[50] Surface charge of the bacterial cell wall governs adhesion of the nanoparticles to bacterial cells. Since these IONPs act only when they come in contact with the bacterial cell walls. There are various means which promotes IONPs-bacterial contact, such as electrostatic attraction, Vander Waals forces, receptor-ligand and hydrophobic interactions. IONPs can cross microbial cell membranes, interfere in metabolic pathways and induce changes in membrane shape and function after coming in contact with the bacteria. IONPs interact with the microbial cellular machinery for the inhibition of enzymes, deactivation of proteins, induction of oxidative stress and electrolyte imbalance, and also modify gene expression levels when it is inside the microbial cell.[53]

Oxidation of IONPs releases of positive ions such as Fe^{2+}, Fe^{3+} can adsorb on the cell surface of bacteria and leading to damage of cell wall by forming of holes in most Gram-negative bacteria. There is change in efflux and influx of biomaterials from bacteria due to electrostatic interaction between the positive charge of iron oxide metal ions and the negative charge of cell envelopes. Blistering (blebs), clumping of membranes, and blockage of electron transport chain, protein and nucleic acid damage, leakage of bacterial cytosol are the other interactions of IONPs with bacteria. Ten to twelve nanometers IONPs binds to sulfur-containing amino acids specifically to cysteine, methionine and homocysteine in the inner surface of bacteria. High affinity is seen in cysteine with the thiol functional group for attachment of metal ions. There will be the change in enzyme function such as NADH dehydrogenases, due to the attachment of cysteine inside the bacteria.

Higher activity of IONPs resulted in the formation of Reactive Oxygen Species (ROS), this is another antibacterial aspect of IONPs. The four types of ROS are superoxide radical (O-),

hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2). For the production of ROS, the active Fe ions such as Fe^{2+}, Fe^{3+} reacts with H_2O_2. The property of photocatalytic have effect on antibacterial activities in the case of IONPs. IONPs photocatalysis is due to electron-hole formation by electron excitation from the valence to conduction band under light or UV radiation. ROS is produced by reaction between electron holes with H_2O molecules. Oxidative stress alters the bacterial membrane permeability and damages the cell. IONPs surface properties involve a high surface to volume ratio and low degree of crystallinity which are effective factors in the rising of antibacterial activity.[15,53]

- **Antibacterial activity of IONPs**

One of the important reasons for antibacterial activity is the release of Fe^{2+} ions. The antibacterial activity of iron oxide nanoparticles depends on mixture concentration, pH, size, distribution and agglomeration of nanoparticles. If the suspension of powder metal oxide is concentrated enough, then there will be larger specific area and the antibacterial activity will be better. The IONPs synthesis parameters, microbial cell wall structure and degree of contact with the bacterial cell, these are the factors that induce the sensitivity of the bacteria to metal oxide nanoparticles. IONPs have several modes of action (e.g., ROS, oxidative stress) as antimicrobial agents. There is also simultaneous occurrence of major lethal pathways. Generally, most IONPs exhibit microbicidal properties by producing ROS or releasing Fe^{+} ions. Fe_3O_4 showed higher antibacterial activity against *E. coli* and *S. aureus*.[52] The antimicrobial activity of Fe_3O_4 nanoparticles against uropathogens such as *S. aureus*, *E. coli* was observed. Chen *et al* demonstrated that immunoglobulin G-bound Fe_3O_4 nanoparticles inhibit various pathogenic drug resistant uropathogen growth such as MRSA.[54,55] The antibacterial IONPs interact electrostatically with the bacterial cell membrane which causes disruption of the membrane.[56]

Chitosan-coated IONPs shows higher antimicrobial activity than naked IONPs, this is due to the positive surface potential of chitosan-coated IONPs. It strongly interacts with polyanionic bacterial surfaces of the cell. The small size of IONPs contribute to antimicrobial activity, also it is useful for accomplishment of antimicrobial actions and killing intracellular bacteria. Fe_3O_4 nanoparticles showed antibacterial effect on the properties of growth and of ampicillin- and kanamycin-resistant *E. coli* strains membrane activity.[19] Also, Fe_2O_3 nanoparticles showed zone of inhibition and maximum sensitivity against MRSA, *E. coli* and *Enterobacter spp*.[32,17] IONPs also showed antibacterial activity against *P. aeruginosa* and *E. coli*.[57]

The effective antibacterial activity was seen against Gram-negative bacteria specifically *E. coli* and other uropathogens like *K. pneumoniae*, *P. aeruginosa* were less sensitive to IONPs in comparison to *E. coli*.[58] IONPs-based nanosystem's incorporated into medical devices showed promising results with regard to the microbial colonization inhibition. IONPs-amoxicillin nanosystem's minimum inhibitory concentration (MIC) on *S. aureus* and *E. coli* planktonic (free flowing) cells showed 3-4 times lower growth than the antibiotic alone. The coating of essential oils-loaded IONPs on catheter surfaces showed decrease in initial adhesion of *S. aureus* and *K. pneumoniae* and lesser effect on more mature biofilm formation stages.[59]

5. Biological Compatibility of IONPs

Biological compatibility/biocompatibility of nanomaterials is their ability to function in a host system and produce an appropriate host response in a specific situation. For the treatment of infectious diseases modified or engineered IONPs are used, this has been possible because of advancement made in the field of nanotechnology. In biomedical applications, IONPs biological compatibility has to be established prior using it.[60] Intensive exploration of IONPS has been carried out. It plays a significant role in targeted drug delivery and diagnostics, that is why prior to their application, histocompatibility evaluation is required. Some system can trigger various biological effects that can be either beneficial or destructive, for e.g., direct contact with cells, tissues, extracellular environment and physiological systems. IONPs biological compatibility and their efficacy is determined by the physiochemical properties which they possess. Therefore, there is a need to establish standard evaluation criteria for biocompatibility which is required for the IONP biomedical applications. Studies of biological compatibility of IONPs can be germane to genotoxicity, cardiotoxicity, hemocompatibility, histocompatibility, neurotoxicity and cytotoxicity. As drugs IONPs can gain access to the body by inhalation, intravenous injection, oral ingestion and contact with the skin. During drug delivery IONPs is used as vectors and they come in direct contact with the blood. Hence, prior to use of IONPs, it is essential to evaluate blood-IONP compatibility. For the evaluation of blood-IONP compatibility various techniques are used such as hemolysis, blood cell aggregation and coagulation behaviour studies. There are several factors on which Blood-IONP compatibility is depended on viz. IONPs size, structure and surface properties of the nanoparticle.[61] IONPs biological effects, activity, their toxicity, and mechanism of action can be understood by pre-screening tool such as cell culture assays.

Toxicity studies reveals that superparamagnetic iron oxide nanoparticles (SPIONs) do not exhibit any severe toxic effects and hence they are biocompatible.[62] Ferrite nanoparticles like Fe_3O_4 showed low toxicity against HeLa cell lines.[63] There are number of studies carried out on nontoxic and biocompatible behaviour of SPIONs in different human and animal cells. In vivo behaviour of SPION in rat liver concluded that it did not influence liver function or induce oxidative stress.[64] Sodium oleate-coated IONPs showed good biocompatibility.[65] Prior IONPs applications are available for human use, the in vitro assays must be confirmed by in vivo studies carried out in animal models even if these results are promising. It is necessary to determine risk assessment of nanoparticles toxicity studies. Hence, for the evaluation of the biological compatibility of IONPs in a tissue and application, more research is necessary in a specific manner.[55,66] Surface functionalization of IONPs is necessary for the stabilization of IONPs, prevention of the undesired particle agglomeration, to optimize bio-interactions and to provide biocompatibility and monodispersibility. Through slight variations in their surface structure of IONPs, even cytotoxic IONPs can be converted into biocompatible materials. Therefore, it may be concluded that IONPs possess a vast level of biological properties that depends on their size, structure, quantity, and receptor cell type.[56]

6. Biodegradability and Encapsulation of IONPs

Biodegradability commonly refers to the internal digestion and following the removal of nanoparticles from the body. Biodegradable IONPs are nano-sized materials which can naturally degrade under biological conditions in the body. Its removal from the body has highly significant function. These IONPs can be prepared from proteins, polysaccharides and synthetic biodegradable polymers. Depending on the size, shape and composition or nanoparticle's structure, different nanoparticles have different encapsulation efficiency. The nanoparticles designs are improving continuously for enhancing biodegradability. Nowadays discovery of revolutionized medicine is due to advances in the development of biodegradable IONPs. Biodegradable IONPs are appropriate for biomedical applications than non-biodegradable IONPs. This is because non-biodegradable IONPs shows more toxic side-effects due to its accumulation in mononuclear phagocytic cells found in the liver and spleen. Polymer-based IONPs are submicron in size, colloidal particles and it can be harboured as a therapeutic agent by encapsulating it within their matrix of the polymer and adsorbed or conjugated onto their surface. There has been increase in the application to the site-specific delivery of vaccines, drugs and other bioactive molecules due to biodegradable IONPs

features which includes their bioavailability, capabilities of high encapsulation, low toxicity, suitability for the controlled drugs release, etc. The selection of matrix material depends upon size of IONPs, characteristics of surface, degree of biocompatibility, biodegradability and toxicity.[51] Several methods for preparation of the biodegradable IONPs include dispersion of preformed polymers (solvent evaporation, solvent diffusion method), ionic gelation method for hydrophilic polymers and polymerization of monomers.

Biodegradable polymer matrices are used in the preparation of biodegradable IONPs such as chitosans, poly-ε-caprolactones (PCL), gelatins, poly-D, L-lactide-co-glycolides (PLGA), polylactic acids (PLA), and poly-alkyl-cyanoacrylates (PAC). Hydrolysis of PLGA in the body leads to production of metabolically biodegradable products like lactic and glycolic acids. In the development of nano-vaccines, gene delivery systems, and protein and peptide-based nanomedicines PLGA IONPs are used. Under normal physiological conditions PCL undergoes hydrolysis with minimal or no toxicity, with applications in long-term implantable devices and drug delivery. PLA breaks down in the body to produce lactic acid and it is considered as a biocompatible and biodegradable polymer. Gelatin IONPs are used in drug delivery and the controlled drugs release. They are nontoxic, biodegradable, and bioactive. Several toxic compounds that damage or stimulate the central nervous system are produced by PACs upon biodegradation. Hence, the use of PAC polymers in humans is not approved. Starch is a natural water absorption polymer which has features of biocompatibility, biodegradability, and non-toxicity. It can be also used as an effective capping agent. Starch containing Fe_3O_4 NPs grafted with hydrogel polymeric networks prevent nanoparticle aggregation, and loaded with heteropoly acid for delivery of the drug.[67]

Macrophages or phagocytic cells are a crucial part of the mono-nuclear phagocytic system and are involved in the clearance of foreign particles which also include IONPs from the circulation. The surface modification of IONPs allow them to escape the immune system of the body. For a prolonged period, surface-modified IONPs which are modified with biomolecules, remain in the vascular system. Hence, surface-modified IONPs can safely and rapidly reach their target site compared to non-modified IONPs.[68] Poly-ethylene-glycol (PEG) is used to modify the nanoparticles surface to enable long-term circulation. Incorporation process of PEG onto nanoparticle surface is known as PEGylation. For the reduction of immunogenicity and toxicity, prolong circulation time, change bio-distribution and optimize nanoparticle activities PEGylation of IONPs can be used. Also co-polymers of

hydrophilic PEG and hydrophobic PCL can yield high biocompatibility and biodegradability, this will make them favorable candidates as nanodrug delivery systems.[69] PLGA-PEG with high biocompatibility and biodegradability developed by the conjugation of COOH–PEG–NH_2 to PLGA–COOH. Then, the anti-tumor drug doxorubicin (DOX) and Fe_3O_4 magnetic NPs encapsulated by PLGA-PEG matrix endow the nanocarriers with anti-cancer and T2-weighted magnetic resonance (MRI) capability.[70,71] Tocopheryl polyethylene glycol 1000 succinate-modified IONPs exhibit increased adhesion towards tumor cell surfaces. A nano-suspension of paclitaxel which is an anticancer agent bound to biologically compatible proteins like albumin (Abraxane) approved for the breast cancer treatment. The benefit of using polymeric nanoparticles is to allow bioactive molecules encapsulation and protect them against enzymatic and hydrolytic degradation.[68]

7. IONPs and Delivery Systems

The effective delivery of a therapeutic compound to the target site is one of the major stumbling blocks associated with the treatment of infectious diseases. Poor bio-distribution, limited effectiveness and lack of selectivity are some of the drawbacks and limitations of traditional approaches to drug delivery. To overcome these drawbacks and limitations of traditional approaches, controlled drug delivery systems are used. The role of these systems is to transport the drug or therapeutic compound to the site of action, protect the drug from rapid removal or degradation from the body and reduce side effects which are undesirable. IONPs have received great interest due to their unique magnetic performance, excessively low cytotoxicity, excellent biocompatibility and biodegradability, and various reactive sites for contact with drugs after being modified with various coatings.[67] The site-specific or targeted drug delivery can be achieved by attaching the therapeutic drug to the IONPs, which act as carriers, known as nanocarriers.

Nanocarriers are used to deliver therapeutic or bioactive compounds. These nanocarriers are absorbed by cells due to its optimized physicochemical and biological properties. For the development of drug delivery systems various nanocarriers are available which includes liposomes, dendrimers, polymers, carbon materials, solid lipid NPs (SLNs), silicon, and magnetic NPs like IONPs. When iron oxide–based carriers are designed and synthesized as nano-carriers there are some important factors which should be considered. These factors include the particle size and its distribution, concentration, colloidal stability, in vivo drug release kinetics, drug loading, intrinsic carrier toxicity. The IONPs magnetic property enable

their remote control using a magnetic field. Therefore, by applying a localized external magnetic field to the targeted site, IONPs can guide to targeted site inside the body, which leads to efficient accumulation of magnetic nanoparticle in tissues or organs of interest. When a drug or therapeutic agent is conjugated (attached, adsorbed or encapsulated) to a magnetic vehicle/carrier, administrated and then released is known as magnetic drug delivery. For different purposes, magnetically guided drug delivery systems have been widely used. The selectivity in delivery and cellular internalization can be enhanced by modifying IONPs surface by special moieties such as peptides, antibodies, and small molecules, that may be recognized by the target cells.[72,55]

SPIONs in aerosol droplets improves magnetic efficacy of targeting, allowing for a localised increase in temperature and facilitation of its transport through the mucus in urogenital systems. For instance, SPIONs were able to control *P. aeruginosa* growth of biofilms. Superparamagnetism allows IONPs to deliver drugs to target specific site with minimal exposure to other healthy cells, under the influence of an external magnetic force. Importantly, in the treatment of UTIs, IONPs under the application of an external magnetic force can act as nano-knives, penetrating the thick mucus and layers of biofilm, exposing the inner layer of uropathogens to delivered drugs at the target site. Some previous studies demonstrated that under the application of a magnetic field magnetic IONPs were able to promote an antimicrobial effect in biofilm matrixes, which causes several bacteria to detach, such as Methicillin-resistant *Staphylococcus aureus* (MRSA). Alginate-coated and tobramycin-conjugated IONPs showed inhibition against *P. aeruginosa* growth and the biofilm formation.[73] To aid therapeutic efficacy by protection of drug, prolonged residence time, and nidus-targeting ability PLGA IONPs are designed for drug delivery.

Frequently, for transporting antibacterial drugs lipid-based nanoformulations, such as liposomes, nanoemulsions, and SLNs are applied. These can fuse with membrane of the bacteria, delivering antibiotics directly to uropathogenic bacteria. Mammalian cells, tumor cells and microbes fused by liposomes can facilitate the transport of drugs across biomembranes. Due to the very low cytotoxicity relative to polymeric nanoparticles, lipid nanocarriers such as SLNs, nanostructured lipid carriers (NLCs) and nanoemulsions, appear suitable as drug-carrier systems. Nanoemulsions are nanocarriers with pure liquid oil in the inner phase. UTIs are treated by targeting bacteria and diminishing biofilm, using these lipid nanocarriers introduced as antibacterial drug carriers.[74] The fabrication of chitosan-modified

IONPs, and then ciprofloxacin as a model of the drug was loaded on the nanoparticles surface by hydrogen bond and/or electrostatic interactions. The loading of ciprofloxacin of approx. 99% was noticeably high, that allowed optimizing the therapeutic effect by reduced dosing and counteracted the side effects and toxic effects.[67] In developing novel nanosystems for increased antibiotic activity aminosilane modified IONPs can be used as polyene antibiotics nanocarriers.[75]

In gene delivery or as biosensors, IONPs can also be used as vectors. Most microbial genomes undergo point mutation and recombination, this will lead to the emergence of new resistant strains. Because of this available, drugs become ineffective and makes the treatment of infectious diseases more difficult. During these situations antisense technology can be used. Peptide analogues of nucleic acids (PNA) form complementary base pairs with RNA, inhibiting gene expression in vivo. PNA delivery into the target site shows inhibition towards the expression of a target gene. IONPs labelled with fluorescent markers, for e.g., Cy-5, helps to visualize the uptake and accumulation of nanotubes.[55]

Drug delivery with IONPs allows to pre-determine drug kinetics and targets which are vital for maintaining optimal dosing within the therapeutic window by using drug in lower amount and this decreases the toxicity and cost of the pharmaceutical formulation. Furthermore, administration of IONPs can be done via a plethora of various routes ranging from local to systemic administrations for achieving the targeted and improved pharmaceuticals delivery, most likely improving bioavailability or allowing for sustained release of drug or prolonged exposure of drug.

8. CONCLUSIONS

Drug-resistant uropathogens gave rise to a great challenge for clinicians to find permanent cure for UTIs and also to lower its occurrence rate. Various mechanisms are used by pathogens to develop drug resistance. Advancement in nanomedicine offers many techniques by using IONPs, to overcome these challenges. IONPs can be used for bioimaging and early detection systems, the diagnosis and treatment of infectious diseases such as UTIs, caused by drug-resistant uropathogens. Researches developed IONPs and their antimicrobial activity has been tested on uropathogens. The green synthesis of IONPs reduces the environmental concerns allied with chemical synthesis which uses toxic chemicals.

Due to outstanding physicochemical and biological properties of IONPs, enormous applications have been found in various other fields of biomedicine such as bioimaging, diagnostics, therapeutic agents, targeted drug delivery, gene delivery, cancer therapy, etc. These nanoparticles can be an alternative over traditional drugs to overcome bacterial resistance, this is due to their antimicrobial activity against Gram-positive and Gram-negative bacteria. Magnetic IONP-based platforms are emerging alone or in combination with antimicrobial agents.

IONPs uses mechanisms of action that differ from the classical treatments. This will have a great advantage of being active against uropathogens that have already developed resistance against drug. It also targets multiple biomolecules which compromises of the resistant strain development. The mode of action of IONPs varies with the type of bacteria. IONPs inhibit the growth of uropathogens like UPEC, *P. aeruginosa*, *S. aureus.* Surface modification of IONPs can reduce its toxicity and can greatly improve the biocompatibility. Therefore, they can be surface engineered and modified with different coating materials (natural or synthetic polymers), moieties and biomolecules to enhance their biocompatibility and stability in biological fluids. IONPs are biodegradable and can be encapsulated, as they can be efficiently implanted in the body and removed when their purpose is achieved. With the use of modern nanotechnologies in drug delivery, the impact of traditionally used antibiotics can be maintained and improved by drug targeting as well as localised high drug concentrations delivered to the urinary tract and to the uropathogens.

Magnetic IONPs showed great results in medicinal field and healthcare treatment due to their low toxicity, good biocompatibility and ability to be manipulated by magnetic field applications. Hence, due to their enormous therapeutic potential and various properties, IONPs becomes an imperative and can be considered as a novel inhibitory weapon against drug resistant uropathogens causing UTIs.

9. Future Prospects

IONPs or its nanocomposites have provided some hope to manage and treat many infectious diseases which would have been difficult by currently available techniques. Prior to safe and effective use of IONPs, there are certain issues that need to be addressed. Such problems include a more detailed understanding of IONPs mechanism of action, development of various eco-friendly methods for their synthesis, environmental and social implications of their use. In biomedical applications, the future of IONPs holds great promise specifically in

the area of disease diagnosis, cellular and deep tissue imaging, early detection, drug and gene delivery as well as multifunctional therapeutics. Incorporation of nanomaterials, especially IONPs, could extend the theragnostic (combines therapeutics with diagnostics) platform construction. This would make the diagnosis processes simpler, speedier and less invasive. The pharma industries usual business model may change in coming years as for biomedical applications the attractive material would be multifunctional IONPs. Nonetheless, for clinical application, the comparison of antibacterial effect of the IONPs with a positive control antibiotic approval is suggested.

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