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Historical perspective

Emerging nanotechnology based strategies for diagnosis and therapeutics of urinary tract infections: A review

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ABSTRACT

At present, various diagnostic and therapeutic approaches are available for urinary tract infections. But, still the quest for development of more rapid, accurate and reliable approach is an unending process. The pathogens, especially uropathogens are adapting to new environments and antibiotics day by day rapidly. Therefore, urinary tract infections are evolving as hectic and difficult to eradicate, increasing the economic burden to the society. The technological advances should be able to compete the adaptability characteristics of microorganisms to combat their growth in new environments and thereby preventing their infections. Nanotechnology is at present an extensively developing area of immense scientific interest since it has diverse potential applications in biomedical field. Nanotechnology may be combined with cellular therapy approaches to overcome the limitations caused by conventional therapeutics. Nanoantibiotics and drug delivery using nanotechnology are currently growing areas of research in biomedical field. Recently, various categories of antibacterial nanoparticles and nanocarriers for drug delivery have shown their potential in the treatment of infectious diseases. Nanoparticles, compared to conventional antibiotics, are more beneficial in terms of decreasing toxicity, prevailing over resistance and lessening costs. Nanoparticles present long term therapeutic effects since they are retained in body for relatively longer periods. This review focuses on recent advances in the field of nanotechnology, principally emphasizing diagnostics and therapeutics of urinary tract infections.

1. Introduction

Infectious diseases topped the list of leading death causes at the beginning of 20th century. Urinary tract infections (UTIs) refer to the presence of microbial infection within urinary tract and classified by the site of infection as cystitis, pyelonephritis and prostatitis. There was no proper widely accepted definition for recurrent urinary tract infections (RUTIs) until 2000 [1]. Then after, many publications on RUTI defined as three or more episodes of UTI in the last twelve months [2,3]. Some publications defined as UTI relapsing two or more times in last six months [4,5]. UTIs are one of the most neglected but common infections, estimated to be nearly quarter of the entire healthcare associated infections. The incidence and severity vary with age, sex and several genetic susceptibility factors. The uropathogens are common all over the world. Both Gram positive and Gram negative have the potential of causing UTIs. Most commonly, Gram negative uropathogenic bacteria include *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *K. oxytoca*, *Salmonella paratyphi*, *Citrobacter freundii*, *Vibrio cholera*, *Serratia marcescens*, *Providencia stuartii*. Enterobacteriaceae constituted more than 80% of the reported cases, of which *E. coli* is the most common pathogen in community acquired as well as catheter associated UTIs [6].

One of the recent technological advances to concentrate on this problem is to explore nanoparticles, against which the pathogens may not develop resistance. Nanotechnology is at present an extensively developing area of immense scientific interest since it has diverse potential applications in biomedical field. Nanotechnology may be combined with cellular therapy approaches to overcome the limitations caused by conventional therapeutics. Colloidal zinc oxide nanoparticles were shown to exhibit antibacterial properties against a broad range of

Abbreviations: UTI, urinary tract infection; RUTI, recurrent urinary tract infection; UPEC, uropathogenic *Escherichia coli*; CDC, Centers for Disease Control and Prevention; NHANES, National Health And Nutrition Examination Survey; NIDDK, National Institute of Diabetes, Digestive and Kidney Diseases; ED, emergency department; CNF, cytotoxic necrotising factor; IBC, intracellular bacterial communities; QIR, quiescent intracellular reservoirs; TMP-SMX, trimethoprim-sulfamethoxazole; IMC, isothermal microcalorimetry; CFU, colony forming units; FTIR, Fourier-Transform Infrared spectroscopy; UVRR, UV Resonance Raman spectroscopy; SERS, Surface Enhanced Raman Spectroscopy; MALDI-TOF, Matrix Assisted Laser Desorption Ionization Time-of-Flight; MS, mass spectrometry; PCR, polymerase chain reaction; rRNA, ribosomal ribonucleic acid; ELISA, enzyme linked immunosorbent assay; Ig, immunoglobulin; LAL, limulus amebocytes lysate; LPS, lipopolysaccharide; SPR, surface plasmon resonance; NP, nanoparticles; UV, ultraviolet; PPE, paraphenyleneethynylene; GNP, gold nanoparticles; PECA, poly(ethyl-2-cyanoacrylate); PAMAM, polyamidoamine; TEM, transmission electron microscope; ROS, reactive oxygen species

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pathogens [7,8]. Zinc oxide nanoparticles offered higher bactericidal activity against *Staphylococcus aureus* compared to other metal oxide nanoparticles [9]. Gold nanorods were employed for specific targeting towards pathogenic microorganisms and destroying them by the use of phytothermal treatment [10]. Gold nanoparticles are higher photostable, lesser toxic, easily attuned to surface modifications compared to silver. Moreover, gold nanoparticles could be modified so that they can absorb near infrared radiations, which could be transferred to the attached bacterial cells in the form of heat and result in irreversible cellular damage [10].

2. Urinary tract infections (UTI)

2.1. Etiology and epidemiology of UTI

UTIs comprise about 13% of the total healthcare associated infections, reported with an estimated number of 93,300 infections annually [11]. It is estimated that more than 1 million patients join hospitals due to catheter associated UTIs, the most common nosocomial infection. As per the reports by the Centers for Disease Control and Prevention (CDC), UTIs account for 13,000 deaths annually, with a mortality rate of 2.3% and it may be increased to 10% if UTIs are associated with bacteremia. 15–48% death rate had been reported in the patients suffering from proteus bacteraemia and 35% of the nosocomial infections reported are due to UTIs [12], and the prevalence of UTIs increases with age [13] and also with the increase in duration of catheterization [14]. Of the patients infected with UTIs in the hospitals, 50% would have a chance of recurrence in the next sixty days. UTI stands among the major three prevalent infections in India [15] and are the most common bacterial infections in infants and children [16]. According to previous studies, 50% of women will suffer from UTI at least once during their lifetime; 10–20% of women experience each year, 1–2% of women encounter with frequent recurrences [17] and 50% of women suffering from asymptomatic bacteriuria are thought to develop pyelonephritis [18]. High morbidity and long term complications like chronic renal failure are associated with pediatric urinary tract infections [19]. A connection between UTI and diabetes mellitus had been identified earlier in the 1940s and urinary tract had been reported as the major site of infection in diabetic patients [20]. UTIs are more frequent in diabetic patients as hyperglycemic urine stimulates bacterial growth and colonization [21]. UTIs are the most common type of infections during pregnancy where cystitis can be rapidly transformed into pyelonephritis [22]. According to NHANES (National Health And Nutrition Examination Survey) and NIDDK (National Institute of Diabetes, Digestive and Kidney Diseases), during 1988–1994 in US, approximately 6.2 million adults or 2.28% had reported suffering from cystitis for more than 3 months. According to Hospital Episode Statistics, Department of Health, England, 0.096% of hospital visits were associated with cystitis in England during 2002–03, 97% of which required hospital admission. Cystitis is estimated to affect 10/100,000 of the Finland population. Approximately 8.2% of the people are estimated to be suffering from prostatitis [23].

The overall hospital mortality rate due to severe sepsis was 28.6%, which represents 215,000 deaths nationally. Patients with preexisting medical conditions would experience higher mortality rates. The mortality rate due to acute pyelonephritis is higher when associated with early clinical failure and the important issues for rapid clinical failure are chronic liver diseases, diabetes mellitus, and malignancy [24]. 15–17 cases were reported per 10,000 populations in the United States and 80% of cases were women [25].

2.2. UTI related healthcare costs

The health care costs can be directly correlated to the economic burden of that specific disease. The economic burden of a disease is the total price of the resources used or lost by the patients, organization and the society due to that disease. It includes direct costs (such as diagnosis and treatment costs), indirect costs (such as the salary lost by the patients, productivity lost by the organization and mortality costs) and impalpable costs (such as costs of suffering). The exact economic burden may be difficult to determine. The total health care costs can be separated into the following five groups: physician visits, inpatient hospitalizations, outpatient visits, emergency department visits and drugs associated with the disease [26].

The total costs for UTIs were thought to be $2.14 billion in 2000 and approximately $2.9 billion in 2013. It has been reported that 15% of the antibiotics prescribed in the USA are for UTIs, the cost of which exceeds $1 billion annually. A study conducted on 1118 emergency department (ED) patients and 41,605 outpatients reported that the mean pharmacy costs were $2971 and $1882 for ED patients and outpatients respectively during the full treatment period [27]. In the UK more than 320,000 patients develop UTIs each year, which results in more than £900 million cost. The Agency for Healthcare Research and Quality reported that sepsis is the most costly disease in U.S. hospitals, with more than $20 billion in 2011.

2.3. Virulence factors

Genetic and immune factors of the host as well as virulence factors of pathogens contribute to UTI. Specific virulence factors residing on uropathogenic *E. coli* membrane play a significant role in attachment to the host cells, colonization, infection, and resistance mechanisms. Adhesins present on the surface of the bacterial membrane act as specific recognition molecules and aid in the initial attachment or adherence of bacteria to uroepithelial cells. The capacity of adherence is the main characteristic of pyelonephritogenic strains of *E. coli* [28]. TolC protein, an outer membrane protein has a role of transferring the α-hemolysin toxin out of the *E. coli* outer membrane [29]. α-hemolysin mediates pore formation on erythrocytes, leukocytes, endothelial cells and renal epithelial cells. Cytotoxic necrotising factor1 (CNF-1) may be involved in polymorphonuclear phagocytosis and apoptosis of uroepithelial cells. Some strains of uropathogens express *S*-fimbriae, the binding sites of which are present on epithelial cells of glomerulus, proximal and distal convoluted tubules and collecting ducts in kidney. Cytolysin secreted by *Enterococcus faecalis* enhances its virulence and is associated with higher mortality. Human erythrocytes are susceptible to cytolysin mediated hemolysis [30]. Endotoxins, also known as lipopolysaccharides present in the outer wall of Gram negative uropathogens are considered to be the main causative agents of the UTIs. Endotoxins are composed of mainly three regions: O-specific antigen which is the main recognition site for the cells of immune system, core polysaccharide and lipid A [31]. Endotoxins induce inflammatory responses when they come in contact with the cells of immune system such as monocytes and macrophages and stimulate these cells to release mediators such as tumor necrosis factor and interleukins and produce free radicals [32]. High concentrations of endotoxins result in overwhelming immune responses that may lead to septic shock [33].

2.4. Biofilm formation

A bacterial biofilm is defined as a three dimensional structured group of cells with complex interactions present in an auto-produced polysaccharide matrix that is capable of adhering to both abiotic and biotic surfaces and characterized by genetic diversity and structural heterogeneity [34,35]. Bacterial biofilms are associated with the severity of a large number of chronic infections. Biofilm producing bacteria are resistant to disinfectants and sanitizers [36], antibiotics, host immune defenses and other stresses [37], that makes it difficult in eradicating the associated infections [38]. Biofilms provide a nutrient rich environment that promotes survival and growth of uropathogenic *E. coli* (UPEC), and protects from antimicrobial drugs [39].

2.5. Pathogenesis

The uropathogens initially colonizes in the urethra and makes its passage into the urinary bladder (cystitis) [40], with primary symptom as pain and frequent urination and in severe cases blood is found in the urine (Haematuria). The pathogens ascend into the prostate gland (Prostatitis), usually accompanied by pelvic pain and pressure in abdomen, or into the ureters. Unless an appropriate treatment, the pathogens move to the kidneys (Pyelonephritis), or infect the blood vessels, and considered as a serious threat as the risk of developing various diseases throughout the body increases. The pathogen infection in the kidneys may form aggregates called urinary stones [41] and lead to acute kidney infection [42], the primary symptoms of which may be fever with high temperature, chills and shivering, nausea and vomiting and the severity of the infection may be indicated by cloudy urine, inflammation along with difficulty in breathing (Tachypnea) and higher heart beat rate (Tachycardia) that eventually lead to urosepsis and renal failure [43]. When uropathogens infect blood vessels, they spread throughout the body and can be identified by rashes in case of meningococcemia, diarrhoea, hypotension, change in mental stability, decreased urine excretion in case of sepsis and septic shock which results in multiorgan failure that eventually lead to death, if left untreated [44]. Nonpathogenic strains of *E. coli* are important in the normal intestinal flora of animals, whereas the pathogenic strains of *E. coli* cause UTI.

The infection of UPEC can be depicted in a cyclical form. The bacteria ascend the urinary tract via retrograde mechanism. UPEC secrete type 1 pili that bind to epithelial cells of urinary bladder and invade them by various virulence factors. At the initial stages, the average length of each bacterium is 3 μm and the size of the community doubles each 30 min [45]. They form intracellular bacterial communities (IBCs) inside the uroepithelial cells (Fig. 1). IBCs may contain 10^4–10^5 *E. coli* cells that originate from a single bacterium and pass through various stages to form polysaccharide matrix encapsulated communities [46]. The disruption of uroepithelial cell and maturation of IBCs lead to dispersal of the bacteria to the neighboring cells that may result in a repeated establishment of IBC cycle [47]. The severity and spread of the disease are characterized by repeated cycles of infection. On the other hand, UPEC can form quiescent intracellular reservoirs (QIRs) that contain a few non replicating bacteria enclosed within membrane bound compartments [48]. The QIRs in the transitional cells are sequestered away from host immunity that keeps them viable for long periods (Fig. 1). QIRs have the potential to reactivate and induce an acute infection in the urinary tract [49].

2.6. Multidrug resistance

Many studies have reported increased resistance of UPEC against fluoroquinolones, trimethoprim-sulfamethoxazole (TMP-SMX) and several other antibiotics in both developing and developed countries all around the world [50,51,52,53,54]. Resistance has emerged against highly potent antibiotics even. Various families of antibiotics such as cephems, penicillins, monobactam, quinolones, aminoglycosides, and tetracyclines have been studied. Tabasi et al. [39] reported that UPEC are highly resistant to ampicillin and tetracycline, whereas sensitive to imipenem, meropenem and nitrofurantoin. Piras et al. [55] have reported several mechanisms through which UPEC has become resistant to enrofloxacin in dogs. However, the mechanism in humans has to be elucidated. Ali et al. [53] studied antibiotic resistance patterns of UPEC in Islamabad and found that UPEC is highly resistant to TMP-SMX and least resistant to amikacin. Bouamri et al. [56] reported highest resistance of UPEC to amoxicillin and TMP-SMX and least resistance to amikacin and fosfomycin. Higher resistance to TMP-SMX was reported in Alexandria, Egypt [57] and the same study established a significant correlation between the presence of *pap* gene and gentamycin resistance in UPEC. Phylogenetic group B2 has shown higher susceptibility frequencies to quinolones than other phylogroups [58]. The same study reported higher resistance rates of UPEC in Karnataka, India to ampicillin, cefotaxime and TMP-SMX; and least resistance to netilmicin, gentamicin and nitrofurantoin. Moroh et al. [59] conducted antibiotic susceptibility tests on uropathogens in urine samples collected in Abidjan and reported higher resistance to amoxicillin, tetracycline and TMP-SMX; and least resistant to cefotaxime and netilmicin. Expression of *marA* could be a marker for development of levofloxacin strains of UPEC [60].

However, the last few years witnessed a decline in morbidity and mortality rates due to infectious diseases, mainly due to the introduction of antibiotics [61]. Nowadays, most pathogens have acquired multi

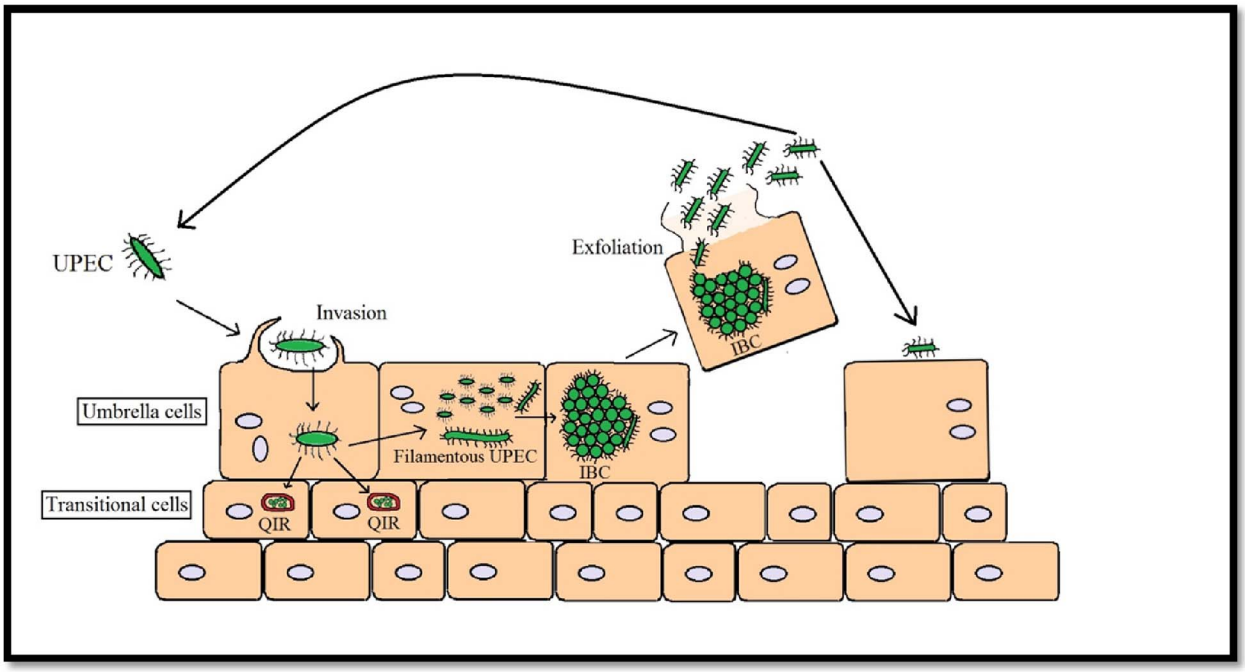

Fig. 1. Principle mechanism lying behind recurrent urinary tract infections.

drug resistance, which may be caused due to the overuse of the antimicrobials. Even though technology is progressing in a rapid manner to alter the existing antimicrobial formulation or to develop new antibiotics; pathogens are ahead in acquiring drug resistance. The emergence of drug resistant bacterial strains demands for a novel long term solution to this rising problem. Day by day, more and more uropathogenic strains are acquiring multi drug resistance against commonly used antibiotics. If this is left untreated, there might come a day when most of the conventional antibiotics would fail in treating infections. Using nanoparticles, against which the pathogens cannot develop resistance, would be a smart choice.

3. Diagnosis of UTI

Diagnosis of UTI starts with acquiring a complete history that includes the previous episodes of UTI, frequency, antibiotic use in the last 6 months, sexual history, spermicide and contraceptive use. Physical examination has to be performed then that includes a thorough examination of pelvic region. Followed by the physical examination, detection of uropathogens in urine samples by various diagnostic approaches mentioned below is generally performed.

3.1. Laboratory based diagnosis

Laboratory based tests are essential for diagnosing UTI, identification and antibiotic susceptibility patterns of uropathogens. Urinalysis serves as the primary means of all laboratory tests in excluding Bacteriuria. The use of these tests minimizes the frequency of redundant antibiotic recommendations.

Urine microscopy was initiated as a clinical practice in 1830, and gained considerable attention through mid-1900s [62]. Microscopic analysis of urine is an essential tool for the management and diagnosis of urinary tract infections. Microscopic analysis of urine is generally performed to check for the presence of bacteria, parasites, yeast, crystals and formed cell structures.

The Sysmex UF1000i is a fluorescence flow cytometer employed for analysis of urine that offers several novel features that are appropriate for microbiological diagnostics [63]. It can perform classification and counting of cells and formed particles like bacteria, red blood cells, leukocytes, yeast-like cells, crystals, spermatozoa, epithelial cells, mucus and small globular cells in unspun urine. It can analyze up to 100 unspun urine samples per hour. This also provides information about the staining characteristics and size of the counted particles. Manoni et al. [64] analyzed one thousand four hundred sixty-three urine samples with the flow cytometer and reported the cutoff values are 125 bacteria/μL (represents 10×10^5 CFU/mL) and 40 leukocytes/μL.

Culturing method is the pioneer technique in bacterial detection, but still being performed as a standard technique. UTI detection from uncomplicated cystitis to complicated pyelonephritis is possible with urine culturing [65]. Clean-catch midstream urine should be collected and culturing must be done within 2 h after collection or refrigerated. The level or severity of the infection may also be estimated by this method [65]. Most of the bacteria require a particular culture medium and specific supplements for their optimum growth. Although it gives accurate results, the time required for the bacteria to grow as visible colonies is 24–48 h. Therefore, culturing method in association with some other uropathogen detection methodology would be beneficial.

Isothermal microcalorimetry technique lies on the principle that the metabolic rate of any living microorganism is directly proportional to the heat released by that organism. The heat released is captured by a thermopile that acts as a heat sensor. IMC was used to assess antimicrobial susceptibility patterns of several uropathogens. This method required 6 h to give accurate results and an extra 1 h for the handling of samples and analysis of data. Minimal inhibitory concentrations of several antifungal drugs on uropathogenic *Candida albicans* in urine were determined using IMC [66]. IMC can work with a wide range of sample volume from nl to L and the time of assay would be 1 h with a detection limit of 10^8 cells per mL [67]. The advantages of IMC include: optical clarity of the sample is not required; the metabolic activity of as few as 10^4 CFU/mL bacteria can be detected. The drawbacks of IMC include: the equilibration of samples is required 1 h prior to analysis to avail high sensitivity; parameters like oxygen depletion have to be included in the results.

Spectroscopic variants such as Raman spectroscopy, Fourier-Transform Infrared spectroscopy (FTIR), and UV Resonance Raman spectroscopy (UVRR) have been used for the detection of UTIs. Raman spectroscopy is based on the plot of scattered light intensity versus energy difference between inelastically scattered photons at different frequencies and incident photons. Raman spectroscopy provides information about the molecular structure and chemical composition of a substance. The major drawback of this method is that it produces very low signals for diluted biological samples often lower than the limit of detection. It produces highly congested spectra, which indicates that it is not highly selective. FTIR is more sensitive than UVRR, but due to the strong absorbance of water at the wavelengths similar to that of biological samples, its use in biological systems is limited. UVRR confers higher sensitivity than Raman spectroscopy and has been employed in the discrimination of uropathogenic bacteria. The highly energetic UV photons may cause photochemical changes in the sample. Extensive use of UVRR is limited due to its high cost and complexity. Surface Enhanced Raman Spectroscopy (SERS) is a modification of UVRR that considerably enhances signal up to 10^{14} times, ensuring simpler, accurate and rapid detection. The signal enhancement is due to Plasmon resonance. SERS spectra offer reduced data congestion, improve the measurement range and can be performed using uncomplicated and relatively economical instrumentation [68].

MALDI-TOF mass spectrometry has become a consistent tool for the detection of bacteria and yeast. The Vitek MS system has received FDA clearance in 2013 for the detection of bacteria and yeast grown on solid media with the exception of *Mycobacterium*. This method provides quick and precise identification of bacteria for most of the urine samples with greater than 10^4 CFU/mL. Vitek mass spectrometry could not identify bacterial concentrations of less than 10^4 CFU/mL. Therefore, MALDI-TOF MS is not capable of detecting bacteria in minute concentrations [69].

Polymerase chain reaction (PCR) is a popular technique used widely to amplify a single or a few copies of specific DNA sequences present in a heterogeneous population to millions of copies with great accuracy in a short time, making possible to detect even minute concentrations of DNA in clinical samples. Several variants of PCR such as Reverse Transcriptase PCR, Real Time PCR, Multiplex PCR and Nested PCR have been developed to detect Bacteriuria, pyuria and most of the uropathogenic components. PCR requires much lesser time to provide accurate results compared to other standard methods. Specific primers or universal primers can be used for the amplification of uropathogenic DNA. Use of universal primers offers an advantage of identifying a vast number of uropathogens in the sample in a single PCR reaction. Most studies use 16S rRNA gene primers for amplification, since it is present in almost all bacterial species. Real-time PCR is considered as the most important variant among all the other variants, as it allows monitoring the formed products simultaneously as the amplification proceeds. Either fluorescent dyes or fluorescent probes are used to visualize the amplification of products [70].

3.2. Immunological based methods

Immunological diagnosis in the field of UTI is of special interest. These diagnostic approaches mainly rely upon affinity. The affinity may be between antigen and antibody or between complementary DNA molecules. Some important immunological based approaches are mentioned below.

Enzyme linked immunosorbent assay (ELISA) is being widely used to identify the uropathogenic components in biological samples. The affinity between the antibodies and antigens is the basic principle behind this technique. Sandwich ELISA is regarded as the most proficient among all other variants of ELISA. The limitations include; each step requires a long incubation period and microtitre plate can accommodate lesser sample volumes [71].

Immunological assays such as latex agglutination, coagglutination, immmunoenzymatic assays are used to detect antibodies and bacterial antigens in biological samples of infected patients. Parry et al. used a slide agglutination test to identify seven diverse antigenic determinants on UPEC fimbriae [72]. Ree et al. performed agglutination reactions using monoclonal antibodies against serologically diverse seven P fimbriae of UPEC to identify the P fimbriae of wild type strains [73]. Plate agglutination test could not detect P fimbriae on wild type strains, whereas coagglutination test could detect the fimbriae on wild type strains. However, the coagglutination test requires only IgG monoclonal antibodies. This study reported that whole bacterium ELISA is the most reliable method among these three methods for detection of P fimbriae on wild type *E. coli* strains. Latex agglutination is simple and sensitive in detecting antigens earlier during infections caused by organisms such as *Haemophilus influenza*. But its use is limited due to the nonspecific agglutination reaction of latex particles. Fein et al. performed yeast agglutination assays to detect UPEC based on the fact that bacteria can agglutinate yeast cells [74]. They were able to detect mannose sensitive UPEC with high specificity and sensitivity. The yeast cells could also be agglutinated by various other Enterobacter species. Ravinder et al. reported that coagglutination test is rapid and cost effective in detection of hydatid antigen in urine samples [75].

3.3. Biosensor based methods

Biosensor based UTI diagnosis is of high demand in the present situation as they are more sensitive than the regular diagnostic methods and are rapid. These diagnostic methods combine biological molecules with electronic output. The biomolecules are sensed through electronic or chemical molecules and provide results in comparatively lesser time [71].

United States approved Limulus amebocytes lysate (LAL) assay in the early 1980s for the parenteral drugs to be tested [76]. It was proved quick, precise and sensitive in endotoxin detection. LAL assay was being used to detect UTIs as gram negative bacteria, the most common uropathogens, secrete endotoxins. Basic principle behind this assay is the natural immune response of *Limulus polyphemus* (horseshoe crab) against endotoxin that results in coagulation of its hemolymph [77]. The hemolymph comprises of 99% granular amebocytes and 1% of non-granular amebocytes. The granular amebocytes are crucial for the defense mechanism against endotoxins. Each granular amebocyte contains large electron dense granules and small granules with lesser electron density. Large granules contain coagulating factors, whereas small granules contain antimicrobial peptides. When endotoxin comes in contact with hemolymph, factor C gets activated. Activated factor C has affinity towards factor B, therefore, interacts and cleaves factor B, activating it. The activated factor B activates proclotting enzyme to coagulase, which interacts with coagulogen. Coagulogen get cleaved by coagulase. This cleavage mechanism is most extensively studied to develop synthetic chromogenic substrates [78]. Coagulogen comprises of three units: A chain, B chain and peptide C. peptide C acts as a connecting link between A chain and B chain. Coagulase acts at the two ends (Arg-Lys and Arg-Gly linkages) of peptide C, joining A and B chains to form an insoluble gel, called coagulin. The concentration of endotoxin in the sample determines the time of gelation [79]. After the gelation is achieved, the concentration of endotoxin can be determined by performing serial dilutions of the sample. This proteolytic cleavage activity is the principle behind Gly-Arg-pNitroaniline chromogenic substrates [78]. Coagulase cleaves the chromogenic substrate to separate *p*-Nitroaniline that is yellow in color and acts as a marker to check the presence of endotoxin [80]. The use of LAL assay for blood samples requires a pre-preparatory step. Erythrocytes contain hemoglobin that interferes with the enzymatic cascade. Therefore, erythrocyte aggregating agent must be added to remove erythrocytes from blood samples [81]. Fungal cells contain β-D-glucan that interacts with factor G in hemolymph to produce coagulation in an independent way [82]. However, several variants of LAL have been introduced by suppressing factor G to enhance the specificity. The detection limit of kinetic chromogenic assay is 0.002 EU/mL with 76 min of detection time [83]. LAL assay has been effectively employed to detect many uropathogens.

Electrochemical biosensors have been successfully employed for detection of uropathogens and their secreted endotoxins. Impedance, amperometric and potentiometric biosensors come under electrochemical biosensors. Recently, an impedance biosensor was developed to detect *E. coli* in urine specimens of humans with a wide range of detection from 70 to 7×10^8 cells/mL [84]. This method has advantages of simple fabrication and easy to use at low-cost. Aptamer based impedance biosensors are also available to detect uropathogen specific DNA. Lipopolysaccharide (LPS) specific aptamer was used to detect LPS with a detection limit of 1 pg/mL and showed no cross reactivity [85]. *E. coli* outer membrane proteins were detected by an impedance biosensor in a label independent manner that had a detection limit of 2×10^{-6} M concentration of outer membrane proteins [86]. Amperometric biosensors are widely used electrochemical biosensors that work on principle of linear correlation between current and concentration of analyte. Lin et al. [87] developed an amperometric sensor based strip to detect *E. coli* O157:H7 with 6 CFU/strip limit of detection in PBS buffer. Campuzano et al. [88] developed amperometric based magnetoimmunosensors to detect *Streptococcus pneumonia* with a detection limit of 1.5×10^4 CFU/mL. LAL based amperometric sensor was developed with a lower detection limit of 0.03 EU/mL [89]. A potentiometric biosensing technique was developed to detect *E. coli* by monitoring the pH changes caused by ammonia, as *E. coli* produces ammonia [90]. An electrochemical biosensor was developed for simultaneous detection of uropathogen and lactoferrin in urine samples. This sensor had the probes specific for most of the common uropathogens [91]. An immunosensor was reported that could detect lactoferrin, a biomarker for UTIs, as less as 145 pg/mL [92]. Zuzuaregui et al. [93] reported a portable biosensor that could detect endotoxins in the range of ng/mL. Toll-like receptor-4 and myeloid differentiation-2 were immobilized on electrodes to prepare a biosensor that could detect endotoxins of gram negative bacteria at concentrations of approximately 0.0002 EU/mL [94].

Higher sensitivity can be achieved by the use of optical biosensors with various phenomena such as scattering and interferometry, and surface plasmon resonance (SPR). An SPR based optical biosensor was used to detect *E. coli* and *S. aureus* using bacteriophages as capturing molecules. This has the potential to detect 10^3 CFU/mL bacteria in 20 min [95]. An optical biosensor that combined luciferase bioluminescence and LAL assay was able to detect endotoxin with a limit of 0.0005 EU/mL in 15 min [96]. Fluorescence phenomenon was also used in biosensing for the detection of LPS by employing green fluorescent protein [97]. A magnetoelastic biosensor was developed to identify the gel formation of LAL assay induced by endotoxins that could detect 0.0105 EU/mL within 20 min [98].

Uropathogen specific aptamers were used to detect specific uropathogens such as *Staphylococcus aureus* [99], *E. coli* [100], *Salmonella typhimurium* [101], *Proteus mirabilis* [102], and other pathogenic microorganisms [101]. Cantilever biosensors monitor the bending of cantilevers due to the binding of target to the analyte present on cantilevers. Cantilever biosensors have been developed to detect LPS of *Hafnia alvei*, a prominent uropathogen [103]. The advantages of cantilever sensors include sensor flexibility, consumption of lesser sample and no need of preparation of samples. The advantages and

Table 1
Advantages and limitations of the diagnostic approaches.

Sl. no.	Diagnostic approach	Advantages	Limitations	References
1.	Dipstick analysis	Lesser testing costs	Lower accuracy and sensitivity	[6]
2.	Microscopy	Pathogens can be observed directly without any pre-preparatory steps.	Low specificity	[104,106]
3.	Flow cytometry	Rapid; shape based and staining based classification of particles	Expensive; requires highly skilled personnel.	[63,105]
4.	Culturing method	Gives accurate results of microorganisms.	Requires greater time; separate culture media is required for various pathogens.	[108]
5.	IMC	As few as 10^4 cfu/mL bacteria can be detected.	Various other parameters interfere with the results.	[107,109]
6.	Spectrometry	Molecular structure and chemical composition of the particles can be determined with sensitivity.	Some forms of photons may cause photochemical changes in sample.	[68]
7.	MALDI-TOF	Quick and accurate identification of bacteria.	Cannot detect bacteria in lesser concentrations.	[110]
8.	PCR	Allows simultaneous detection of bacteria during the test itself.	Some bacteria use specific primers for their amplification.	[70]
9.	ELISA	Since the affinity between antibodies and corresponding antigens is specific, the test offers higher specificity.	Requires longer incubation periods; cannot accommodate larger sample volumes.	[71]
10.	Agglutination reactions	Highly specific as it depends on the interaction between antibodies and antigens.	Cross reaction or non-specific binding to latex particles results in false positive results.	[111]
11.	LAL assay	Natural immune response of horseshoe crab provides the results of the test.	Assay of blood samples requires pre-preparatory step; several particles other than endotoxins gives positive results.	[70]
12.	Impedance biosensor	Uses both potential and current parameters; higher sensitivity.	Poor detection limits; expensive.	[85]
13.	Amperometric biosensor	Uses lesser analyte volumes; higher signal to noise ratio.	Depends on flow rate of analyte.	[112]
14.	Potentiometric biosensor	Lower response time; linear response.	Requires reference electrode; electrodes may be damaged.	[71]
15.	Surface plasmon resonance	Multiplex capability; no electromagnetic interference.	Swelling of matrix may cause path length instability; photobleaching.	[70]
16.	Fluorescence biosensor	Spontaneous; requires no exogenous enzyme substrate.	Fluorophore toxicity.	[71]
17.	Electrochemiluminescence	High signal/noise ratio; background optical signal absent.	Higher sensitivity is achieved only at higher concentrations of ([Ru(bpy)3]Cl2).	[113]

disadvantages of currently available diagnostics for UTI are shown in Table 1.

4. Nanotechnology applications in UTI

In the field of healthcare, rapid and sensitive detection of causal pathogen is the chief aspect to improve patient care, to choose an empirical treatment and to prevent the spread of disease. Hence, various diagnostic approaches have been proposed, each possessing its own pros and cons. Some of them may suffer with lower sensitivity, some with longer reaction time, some with lower specificity, while some are too expensive. Nanotechnology steers clear of major problems and presents enhanced accuracy over current methods. Nanomaterials are attaining huge attention due to their potential for higher selectivity and specificity.

Aluminium nanoparticles when tested against *Escherichia coli* exhibited a mild growth inhibitory activity only at higher concentrations of 1000 μg/mL. Therefore, they cannot act as potent antibacterial nanoparticles [112].

4.1. Effect of nanoparticle size and shape

Several discoveries and studies in the past decade were conducted to demonstrate the optimum size and shape of nanoparticles (NPs) that exhibit highest activity [113,114,115]. The shape and size of nanoparticle influence its catalytic, electromagnetic and optical properties [116,117]. Distinctly shaped silver nanoparticles were inspected for the antibacterial properties against *E. coli*, most common uropathogen [118]. This study demonstrated that trimmed triangular nanoplates with a specific lattice plane offered highest bactericidal activity against the bacteria, compared to other shapes.

4.2. Nanotechnology in antibacterial catheter coatings

Among several forms of UTIs, catheter associated UTIs are the major infections, contributing 80%. Several reports till date have confirmed that no single antimicrobial treatment could eradicate catheter infections to expected levels. In this context, nanotechnology proved its caliber in inhibiting bacterial growth for a period of more than seven days.

Various pathogenic factors influence catheter associated infections, of which the most important is biofilm producing capability of pathogenic microorganisms. Biofilms are frequently seen in such infections. Hence, targeting biofilm might reduce the risk of such infections. Several researchers focused on the development of biofilm inhibitory strategies. In this process, urinary catheters were coated with several substances that showed biofilm inhibitory activity such as nitrofurazone, silver hydrogel [119], furacilinum, furanone [120]. Silver hydrogel and nitrofurazone coated catheters were found effective against all uropathogens except *Pseudomonas aeruginosa*. Catheters coated with oPDM-plus-protamine sulfate that were potent in inhibiting *P. aeruginosa* and *Staphylococcus epidermidis* [121]. Although almost all the catheter coatings provided effective growth inhibitory activity against several pathogenic microorganisms, they failed in providing long-term biofilm prevention after catheter insertion. This led to the recurrence of UTI. Research on molecular mechanism proved that elements like iron are required for biofilm formation. Hence, catheter production without iron has been started and is under trials [122]. The use of probiotics in catheter production has also been evaluated. Catheters when inoculated with non-pathogenic *E. coli* have shown declined rates of pathogen infection [123,124]. However, the efficiency and cost of these catheters remained unclear. Magnesium fluoride nanoparticles were used by Lellouche et al. to coat the catheter surfaces that inhibited the biofilm production by *E. coli* and *S. aureus*. These catheters showed a considerable decline in bacterial colonization over a period of seven days [7]. The same group also investigated the effect of yttrium fluoride

nanoparticles, which also provided enhanced protection [125,126]. JUC, a nanotechnology antimicrobial spray, has also been employed in biofilm inhibition. JUC is a physical spray used for dressing, which forms a positively charged coating that kills or inhibits the growth of all microorganisms that comes in contact with it. In this study, JUC sprayed catheter was inserted into the ureters. JUC was also sprayed on skin, mucous membrane around urethra and the tube attachment point twice a day for 7 days [127]. This method showed considerable reduction in bacterial growth. Cooper et al. coated silver onto the catheters by photo-chemical deposition and were exposed to UV radiation to stimulate the synthesis of silver nanoparticles. These catheters showed biocidal activity that would inhibit the recurrence of urinary tract infections [128].

4.3. Nanotechnology in diagnosis of UTI

Optical imaging to visualize cells or tissues use light scattering by cell membranes, mitochondria and tissues. Discrimination of two different tissues becomes possible when there would be a contrast between different tissues. However, the tissues in the specimen cannot offer adequate contrast to distinguish [129]. Gold nanorods were used as contrast agents since they can strongly absorb and scatter light and hence employed in biomedical diagnosis. The shape of the gold nanoparticle plays a major role in light absorption and scattering since the optical absorption efficiency of gold nanorods is 20 times greater than that of gold nanospheres [130]. Gold nanorods offer the additional advantage that they do not suffer from photobleaching and clearly visible in both optical microscope as well as electron microscope [131]. The unique property of gold nanoparticles to absorb and scatter light can be utilized to demolish bacteria and tissues by heating the surroundings or by releasing substances of therapeutic significance [132]. Magnetic NPs can also be used as contrast agents to observe the affected regions of urinary bladder. Magnetic field has to be applied at the affected regions of the bladder so that the magnetic NPs would localize at those regions. The surface of the NPs can be covered with any substance.

The conjugation of nanotechnology based substances with nanoparticles will ensure precise targeting and quantification of pathogenic microorganisms responsible for the disorder at the single molecule level [133]. The applications of nanoparticles in biomedical diagnostics are diverse and unlimited since nanoparticles facilitate selective targeting of clinically significant targets that include bacteria, DNA, proteins and various biomarkers [134,135,136]. Probes based on gold nanoparticles were reported to detect bacterial DNA with a very low detection limit that 10 fmol of DNA could be detected [137]. *Staphylococcus* DNA as less as 50 fmol was detected by using gold nanoparticles that required hybridization of only 1 h [138]. A study was reported that was able to detect *Staphylococcus aureus* within 10 min using a membrane protein. Gold nanoparticles were conjugated to secondary antibodies, which have tendency to primary antibodies that are specific to membrane protein. Gold nanoparticles served the purpose of detection reagent on immunochromatograph strip and the detection limit of this technique was approximately 25 ng/mL [139]. The color signal can be enhanced by precipitating silver onto the aggregations of gold nanoparticles to make more visible [131].

Gold nano wire arrays along with linker arm attached to specific *E. coli* antibodies were used to detect urinary tract infections. Drug resistant bacteria such as methicillin resistant *Staphylococcus aureus* could be distinguished from non-resistant bacteria using nanoparticles with definite Raman spectroscopic fingerprints. Metabolic activity and antibacterial susceptibility of microorganisms could be evaluated using Con A-conjugated supermagnetic iron oxide nanosensors. Recent studies have proven the practicability of accomplishing rapid and reliable assays for infections in media with no prior sample preparation.

In a technique discovered by Wang et al., DNA of a specific *E. coli* strain, *E. coli* O157:H7, was detected using two different gold nanoparticles of varying sizes: one with 18 nm and the other with 70 nm. 18 nm gold nanoparticles were immobilized onto the surface of quartz crystal microbalance which acted as a stage to bind specifically to biotinylated DNA of the bacteria. This permits the binding of a large number of specific DNA molecules to the surface. Then, 70 nm gold nanoparticles were added to amplify the signal [140]. The sensitivity of this technique was very high compared to the conventional methods, but it suffers with a disadvantage that the bacterial DNA must be altered in order to use this technique [131]. Gold nanoparticles conjugated with poly paraphenyleneethynylene (PPE) were employed to detect bacterial DNA by fluorescence phenomenon [141]. When GNPs are conjugated to PPE, PPE exhibits no fluorescence. However, GNPs release PPE when they come in contact with bacteria due to the electrostatic interactions between bacteria and GNPs. The free PPE show fluorescence, the level of which is determined for the quantification of bacteria [141].

4.4. Nanotechnology in drug delivery

Conventional drug based therapies bear a disadvantage that the drug is dispersed, but not localized to the target site or target cell. Whereas, nanoparticles based therapies overcome this disadvantage by specifically accumulating and targeting at a specific site or target tissues [142]. Diverse nanodrug delivery vehicles have been fabricated over the last decade such as liposomes, polymers, nanotubes, dendrimers and nano rods [143]. Nanodrug delivery offers several advantages: enhance specificity to target cell, alter pharmacokinetics, act as non-toxic carriers, increase bioavailability, facilitate a controlled release of therapeutic substances, increase the solubility of weakly water soluble drugs and most importantly, simultaneous delivery of more than two drugs at a time for combination therapy [144,145].

In the context of UTIs, the uropathogens reside in the urinary bladder and form quiescent intracellular reservoirs in bladder epithelial cells that contribute to recurrence of the infections. Therefore, the therapeutics or drugs must be targeted to bladder specifically. Conventional drugs or antibiotics are not target specific. Intravesical drug delivery is recommended in such cases [146]. Nanotechnology would fulfill this demand of delivering the drug specifically inside the bladder. Polymeric nanoparticles could be used for controlled delivery of drugs into bladder. The level of drug loading and delivery can be managed by altering the chemical nature of nanoparticles and the cross-linking reactions engaged. The drug encapsulated by nanoparticles would be delivered by diffusion or erosion or both [147]. Poly(ethyl-2-cyanoacrylate) (PECA) nano particles loaded with epirubicin were targeted to bladder cells by Chang et al., which demonstrated higher penetration of epirubicin into the bladder walls compared to epirubicin in powdered form [148]. The target of these nanoparticles was highly specific since they caused no damage to urothelial cells. PECA nanoparticles loaded with other drugs that target the uropathogens may be effective in the treatment of UTIs.

The efficiency of Liposomes as nanocarriers of antibiotic has also been explored. Liposomes are lipid bilayer membrane bound spherical vesicles encapsulating aqueous region. Advantages of using liposomes as nanocarriers are: a] they are synthesized from natural components b] they confer no toxicity against mammalian cells c] they do not provoke immune response d] they are biodegradable e] various drugs could be internalized into the liposomes. Liposomes carrying several antibiotics such as amikacin, gentamicin, polymyxin B, Clarithromycin, meropenem have been used to test their potency against various uropathogens, mainly *P. aeruginosa* and *S. aureus* [149,150,151,152,153].

Dendrimers are spherical macromolecules with regular branches and well organized core region that could be explored as nanocarriers in delivering antimicrobials. Although limited literature is available regarding their applications in combating urinary tract infections, they offer some chief advantages. Polyamidoamine (PAMAM) dendrimers were explored mainly in treating tumors. Their well-regulated structure

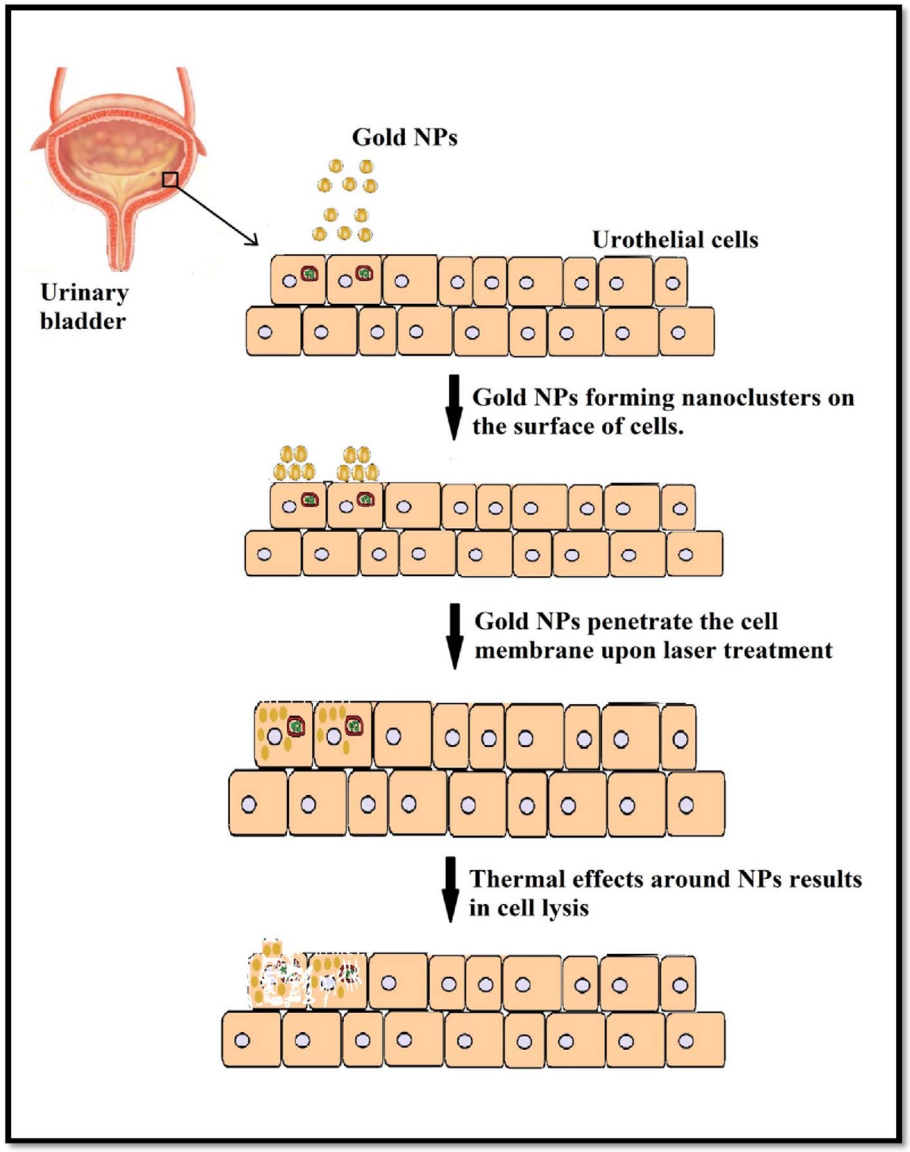

Fig. 2. Gold nanoparticles exhibiting photothermolysis effect.

enables them to flow through the blood vessels and reach the target with ease [154]. It could be expected that they would be explored in the field of UTIs in the near future. The finding of sulphamethoxazole encapsulating PAMAM dendrimers resulted in sustainable release of the antibiotic and enhanced antibacterial activity against *E. coli*.

4.5. Nanotechnology in UTI treatment

Nanomaterials or nanoparticles that exhibit antimicrobial properties by themselves or enhance the efficiency of antibiotic administration are called nanoantibiotics [155]. Nanoantibiotics would not pose any adverse reactions in patients; however, the effect of long term exposure to these nanoantibiotics is still unknown. The synthesis of antimicrobial NPs would be cost-effective compared to conventional antibiotic preparation, along with other additional advantages such as higher stability for longer periods. Antimicrobial NPS compose of metals and metal oxides, antimicrobial compounds, surfactant based nanoemulsions and carbon based nanomaterials. These nanoantibiotics may destroy pathogens by several microbicidal mechanisms: a] they may produce reactive oxygen species that damage the microbial cell components b] they may degrade the cell wall of pathogens c] they may interfere in energy transduction mechanisms d] they may slow down or hinder the enzymatic activity and DNA synthesis [156]. Nanoantibiotics would be more useful in eradicating intracellular infections. Conventional antibiotics, although are effective in inhibiting bacteria, they are least efficient in acting against quiescent intracellular reservoirs. Uropathogens take advantage of this limitation and cause recurrence of UTIs after the effect of antibiotics has been nullified. Nanoantibiotics could be targeted to intracellular reservoirs to steer clear of recurrence mechanisms [157].

4.5.1. Gold nanoparticles

Antibiotics conjugated to GNPs were used to localize the antibiotic effect on the target bacteria. GNPs conjugated with vancomycin were employed against vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium*. The two bacteria have been identified as potent inducers of UTIs. The conjugation with GNPs resulted in 50 fold increase in the activity of vancomycin [158]. These nanoparticles when used in double dose, were able to destroy *E. coli*, the most common uropathogen. Similar studies were reported that showed increased antibiotic activity when conjugated with GNPs. Grace et al. demonstrated the increased activity of antibiotic conjugated GNPs against several Gram positive and Gram negative organisms such as *E. coli*, *S. aureus* and *Pseudomonas aeruginosa* [159]. Saha et al. reported GNPs conjugated with antibiotics, ampicillin, kanamycin and streptomycin against *E. coli* and *S. aureus*. This study demonstrated that conjugation

not only increased the antibiotic activity, but also the heat stability of the conjugated antibiotics [160].

Staphylococcus aureus was targeted by using monoclonal antibodies against a specific surface protein. Gold nanoparticles conjugated with secondary antibodies were employed to bind to primary antibodies [161]. Transmission electron microscopic analysis showed that 40–50 gold nanoparticles were bound to the surface of bacteria forming nanoclusters. The bacterial cells when exposed to laser energy, the nanoparticles present on the surface penetrated the cell wall. Laser energy induced local thermal effects around the nanoparticles, called photothermolysis [162]. The high temperature inducing property of the nanoparticles assisted in physical damage to the bacteria, confirmed by TEM analysis [163].

Huang et al. designed Fe_3O_4 nanoparticles that were in the shape of nanoeggs, were coated with gold onto their surface. These NPs were immobilized with vancomycin, a potent antibiotic to target several uropathogens such as *E. coli*, *S. saprophyticus*, methicillin resistant *S. aureus*. The photothermal effects were analyzed against the bacteria. These nanoeggs attached to the cell wall of bacteria. Magnetic field was arranged to localize the nanoparticles along with the bacteria at one spot to irradiate the NPs and destroy the bacteria. This technique showed a high rate of killing of bacteria by using Fe_3O_4 nanoeggs as photothermal agents [164]. Photothermolysis property could be employed in UTI to destroy the cells containing IBCs or QIRs. Photothermolysis effect is demonstrated in Fig. 2. Vancomycin coated gold and iron oxide nanoparticles were capable of hindering the growth of vancomycin-resistant *S. aureus* and *S. saprophyticus*, both of which are prominent pathogens causing urinary tract infections. GNPs have a lot of significance in diagnosis and quantification of bacteria. Some of these novel ideas could be expected to come into clinical practice.

4.5.2. Silver nanoparticles

Silver, magnesium and iron particles when reduced to nano size were proved to exhibit antibacterial activity against *E. coli* and *S. aureus*. Silver NPs target the respiratory system and cell division of microorganisms that eventually result in cell death [165]. Although prolonged exposure to soluble silver may adversely affect kidney and intestinal tract [166], metallic silver NPs confer a negligible risk to human health and are reported to be non-toxic [167]. However, some studies have reported that silver NPs when used at higher concentrations cause negative effects on mitochondrial activity [168,169]. Thus, the activity of silver NPs must be deeply investigated for their introduction into clinical practice.

4.5.3. Zinc nanoparticles

Bactericidal effects and cell internalization effects of zinc oxide nanoparticles against *S. aureus* were reported by Huang et al. This study confirmed that the bacteria were killed when they came in contact with the NPs. The alteration of nanoparticles surface resulted in higher membrane permeability [170]. Zinc oxide NPs offer more advantages compared to silver NPs in terms of low synthesis cost and blocking UV radiations [171]. The mechanism underlying the ZnO nanoparticle mediated destruction of bacteria is through breaking down the proteins and lipids of bacterial cell membrane [172]. ZnO NPs when coated as a layer onto cotton clothes effectively showed antimicrobial activity against *S. aureus* [173].

4.5.4. Titanium nanoparticles

Titanium dioxide nanoparticles could also be used for destruction of bacterial cells. The antimicrobial activity is based on the photocatalytic property of TiO_2 NPs [174]. Production of reactive oxygen species (ROS) by TiO_2 was also reported [175]. TiO_2 NPs when irradiated with near UV light presented high bactericidal action, which was tested against *E. coli* K-12 bacteria [176], and when irradiated with UV-A showed higher growth inhibiting efficiency against *E. coli* and *P. aeruginosa* but were least effective against *E. cloacae* [177]. B-lactam antibiotic conjugated with nanoparticles showed antibacterial activity against methicillin resistant *S. aureus*. This technique involved the conjugation of antibiotic by emulsion polymerization, which paved the way to conjugate any water insoluble drug onto the nanoparticles [178]. Kuhn et al. reported the antibacterial activity of these NPs among several common uropathogens. They showed the highest antibacterial activity against *E. coli*, followed by *P. aeruginosa*, *S. aureus*, and *E. faecium*, based on the membrane density [179].

4.5.5. Chitosan based nanoparticles

The antimicrobial activity of chitosan based NPs was earlier thought to be effective against viruses and fungi but not against bacteria. The studies further evaluated the efficiency against bacteria. Low molecular weight chitosan was found to inactivate Gram negative bacteria while high molecular weight chitosan was found inactivate Gram positive bacteria [180]. Chitosan conjugated with sulfamethoxazole showed potent bactericidal activity against *P. aeruginosa*. Various mechanisms were proposed to explain the chitosan based destruction of bacteria. Chitosan particles bind to negatively charged bacterial membrane or wall and increases the membrane permeability that leads to outflow of cellular components and death [181]. Chitosan particles inhibit bacterial growth by chelating metal ions that are required by bacteria to perform enzymatic activities [182]. Native chitosan was found least effective in damaging the bacterial membrane. However, water soluble derivatives of chitosan exhibited greater antibacterial activity [183]. Oleoyl-chitosan NPs were synthesized and reported to inhibit *E. coli* and *S. aureus* by damaging the bacterial membrane [184].

4.5.6. Nitric oxide releasing nanoparticles

Nitric oxide and reactive nitrogen species exhibit antibacterial activity similar to reactive oxygen species. However, NPs that could deliver nitric oxide are not available. Research is being carried out in developing suitable and biocompatible NPs that could store and deliver nitric oxide. A few literature is currently available regarding synthesis and efficiency of NPs that could act as vehicles for nitric oxide. Silica NPs were constructed that released nitric oxide, which inhibited biofilm producing Gram negative and Gram positive bacteria such as *P. aeruginosa*, *E. coli*, *S. epidermidis* and *S. aureus* [185,186]. These NPs were

Table 2
Antimicrobial NPs tested in the field of UTI.

S. no.	Nanoparticle	Mechanism/target	Tested uropathogens	References
1.	Silver NPs	Target the respiratory system and cell division of microorganisms; interferes in electron transport system	*E. coli*, *S. aureus*	[167]
2.	Gold NPs	Photothermolysis	*S. aureus*, *S. saprophyticus*, *E. coli*, *P. aeruginosa*, *Enterococcus faecalis*, *E. faecium*	[160,161]
3.	Zinc oxide NPs	Break down membrane lipids and membrane proteins of bacteria	*S. aureus*	[172,174,175]
4.	Titanium dioxide NPs	Photocatalysis; production of ROS	*E. coli*, *P. aeruginosa*, MRSA, *S. aureus*, and *E. faecium*	[176,179]
5.	Chitosan based NPs	Bind to negatively charged bacterial membrane and increase the membrane permeability	*P. aeruginosa*, *E. coli* and *S. aureus*	[187,61,183]
6.	NO releasing NPs	Produce reactive nitrogen species.	*P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis*	[61]

confirmed to be non-toxic against mammalian cells. Silane hydrogen based NPs were reported to be potent antimicrobial agents against methicillin resistant *S. aureus*, which would be helpful in case of sepsis [187,188]. An overview of nanoparticles and their antimicrobial mechanism is represented in Table 2.

5. Conclusion

In the present situation, there is a great requirement for antimicrobial materials across various disciplines, including UTIs. Antimicrobial materials should possess a unique feature that the pathogens should not develop resistance or tolerance against them. They should be able to reach intracellular targets without damaging mammalian cells, or without posing any adverse effects to the host cells. There should be a mechanism of localization at a specific tissue and controlled release of the drug. Owing to their antimicrobial characteristics, nanomaterials can satisfy all the mentioned characteristics with high precision. Additionally, they are stable, cost effective and non-toxic.

Diagnosis of UTIs plays a major role in reducing the severity of these infections. Early diagnosis results in easy eradication of pathogens. Delay in diagnosis results in formation of intracellular reservoirs that are very difficult to eradicate. Conjugation of nanotechnology with currently available diagnostics would be a solution to diagnose UTIs at early stages. With the ongoing advances in the field of nanotechnology, it would definitely overcome the major limitations and stand as the best diagnostic and treatment strategy in preventing infections and their recurrences.

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