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[Review Article]

Diagnosis of Subclinical *Staphylococcal Aureus* Bovine Mastitis Using Nanotechnology-Based Techniques

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Abstract

Mastitis is one of the most common infectious diseases that causes the financial losses for dairy farms. The primary step between determining the etiology of the disease and finding a treatment is accurate diagnosis. Researchers make an effort to develop sensitive, specific, rapid, and cost-effective diagnostic methods for bovine subclinical mastitis caused by *staphylococcus aureus* to solve problems with current diagnostic methods. There are currently new developments in the use of nanotechnology-based techniques for colorimetric, immunological, and molecular detection to design biosensors for the accurate, selective, and functional detection of biomolecules. Nanoparticles are good for replacement and incorporation with other typical traditional subclinical mastitis diagnostic methods due to their nanoparticle size and adaptable physicochemical features including; electrical, electrochemical, optical, and magnetic properties. Hence, the present review discusses the applications and the role of nanotechnology in the diagnosis of subclinical *staphylococcal aureus* bovine mastitis.

Keywords: Subclinical mastitis, Nanotechnology, immunological assays, molecular assays, colorimetric assay.

Introduction

Bovine mastitis, a mammary gland inflammation, is one of the most economically important production diseases that cause severe economic losses to dairy industry [1]. The direct economic damages involve treatment costs, discarded milk as well as costs associated with fatalities and repeated cases of mastitis. Moreover, indirect costs are related to decreased milk production and milk quality besides increased culling rates and animal welfare aspects [2]. In addition, mastitis lowers fertility in cows and causes serious risks to public health [3,4]. The estimated overall cost of failure resulting from bovine mastitis is $147 per cow each year, with culling and losses in milk production accounting for 11% to 18% of the gross margin per cow annually [5]. In Egypt, the prevalence of bovine mastitis was recorded at 46% and the most frequent bacterial isolates were *Staphylococcus aureus* (44.9%) [6,7].

Mastitis can be classified broadly according to observed symptoms into clinical and sub-clinical forms [8]. Clinical mastitis is characterized by a rapid onset of udder redness and edema [9]. In addition, the milk of an inflamed quarter contains particles or clots and/or has a watery consistency with a high somatic cell count , Cows may exhibit visible signs of lethargy [10]. It has been reported that the incidence of subclinical mastitis is higher than that of the clinical form, and it lasts longer because the infection acts as a reservoir for

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pathogens that disseminate the udder infection throughout the herd [11]. Subclinical mastitis, the most prevalent type of mastitis, is an asymptomatic form of intra-mammary inflammation characterized by milk that appears to be normal and an increase in somatic cell count (SCC) as a result of an increase in leukocytes [12].

Mastitis in cows may impair conception and fertility as well as negatively impact the ovarian follicular response [3]. Oocyte competency can be significantly hampered by both subclinical and clinical mastitis, which will reduce the number of produced blastocysts [13]. The most prevalent causes of subclinical mastitis are *staphylococcus aureus* (*S. aureus*), non-aureus Staphylococci and Streptococcus spp [14]. For the dairy industry, a definitive diagnosis of mastitis is essential to guarantee clean milk production, financial returns, public health issues, and animal welfare compliance. Besides, early as well as rapid diagnosis is crucial and should be applied for mastitis management procedures and early therapeutic interventions [14]. To date, diagnosis of subclinical mastitis can only be done using special laboratory tools such as the California mastitis test (CMT), Whiteside test and Somatic cell count (SCC) and Electrical conductivity test. None of the methods listed above can determine the causative agent or the quantitative results for the severity. As a result, microbial isolation and identification are required for a definitive and accurate diagnosis, which is essential to better treatment and control measures [15]. Current methods rely on microbial culture and biochemical assays, are time-consuming, and can detect only viable bacteria. Consequently, false negative results are obtained, leading to increased impairment and production losses [16].

Recently, there has been a lot of interest in developing nanotechnology-based techniques to solve the problems with current diagnostic methods through their specific mode of action and unique physical and chemical properties [17]. These techniques can be used to provide accurate, dependable, quick, safe, cost-effective, sensitive, specific, and easily accessible methods for detecting infections in subclinical mastitis [18]. Nanoparticles can be a future applicant for rapid diagnosis of bovine mastitis pathogen [16]. The application of nanotechnology in immunological and molecular detection is currently undergoing significant advances, and more work is being done to create biosensors that will enable the precise, sensitive, functional, and selective detection of biomolecules [19]. Numerous nanoparticles, including fluorescent nanoparticles, metallic nanoparticles, and magnetic nanoparticles, have been utilized effectively for the diagnosis of subclinical mastitis [20]. Nanoparticles with specific surface modifications can interact with biomolecules like proteins and DNA. Nanoparticles are commonly employed in biosensors nowadays to detect nucleic acids and proteins. These nanoparticles are good for replacement with other typical traditional methods due to their nanoparticle size and adaptable physicochemical features (including electrical, electrochemical, optical, and magnetic properties) [21]. This review will discuss nanoparticles that have the potential to be incorporated into current diagnostic tools for subclinical bovine mastitis.

New developments in the use of nanotechnology for colorimetric assay

Over the last two decades, using nanoscale biosensors as novel analytical tools has been a significant increase. This growth is evidenced by the large number of reports on the use of "ultrasensitive methods," "early detection," "cost-effectiveness," and potential tools for "mass production" that are cited in the Web of Science. Colloidal gold nanoparticles have been extensively investigated because of their special characteristics, which include chemical inertness, surface, high electron density, and, most critically, strong optical absorption [22,23]. The development of an amplification-free visual test that uses numerous gold nanoparticles (AuNPs) collected on a magnetic microbead surface led to the augmentation of the plasmonic signal and an increase in the assay's overall sensitivity, for rapid, and precise identification of microbial DNA in mastitic milk for early diagnosis of infections. This test could be done in 1–1.5 h following post-template DNA production, and results can be seen with the naked eye (no special equipment required) [24].

To create a fast, cheap, and sensitive method for detecting microRNA146a (miR146a) for the early detection of bovine mastitis in the agrifood industry, gold nanoparticles were surface-functionalized with selected RNA probes. The strategy relies on a color change that can be visually observed following the aggregation of the RNA modified-gold nanoparticles (AuNPs) in the presence of miR146a [25].

Mastitis increases plasmin levels leading to an increased proteolysis of milk proteins. Hence, plasmin proteolytic activity plays a key role in mastitis as a biomarker for the detection of bovine mastitis so a simple colorimetric biosensor was developed to detect mastitic milk [26]. A black self-assembled monolayer was formed on a gold sensor surface by the combination of magnetic nanoparticles and plasmin substrate that was broken down by high plasmin levels, and the peptide fragment connected to the magnetic beads was drawn to the magnet beneath the sensor strips, exposing the golden surface. It has been established that this method's in vitro sensitivity was 1 ng/ml of plasmin [26]. According to their analysis, there is great potential

for the created colorimetric biosensor to be used in the diagnosis of mastitis, with possible implications for the food, health and environmental sectors.

A rapid test for detecting mastitis was developed, which involved immobilizing tag-specific antibody molecules and binding double-tagged amplicons, followed by conjugating black carbon nanoparticles with molecules of a fusion protein of neutravidin and alkaline phosphatase on their surface. Antibodies were printed onto three different nitrocellulose membrane slides, and the darkness of the spots was determined using flatbed scanning and visual inspection. This method can detect four mastitic pathogens within a timeframe of less than three hours [27].

Zinc oxide (ZnO) is an inexpensive material that can be produced using a variety of methods that regulate its size, shape, and functionality. Silanes, polyvinylpyrrolidone, amines, and aliphatic thiols are examples of capping agents that are used to alter the material's surface [28]. Because of their special physical and chemical characteristics, ZnO nanostructures are widely employed as multifunctional semiconducting materials for intricate biosensing applications [29,30]. These room-temperature optically stable nanostructures have a broad band-gap and strong exciton energy [31]. The useful application of ZnO for visible-light spectrum fluorescence improvement has been reported [32]. Importantly, ZnO nanoparticles serve as strong signal enhancers for fluorescence -based sensors by absorbing UV light [33]. The evanescent electric field at the nanomaterial's surface, which raises the fluorescent molecule's excitation energy, is one of the mechanisms underlying the fluorescence amplification [29]. By adding tetraethyl orthosilicate-coated zinc oxide nanostructures into a conventional assay, it is possible to enhance fluorescence due to the nanomaterial accumulative near-field effect. This approach had been proven to be effective in real milk samples, allowing clinically relevant concentrations and early detection of subclinical bovine mastitis, so that providing an alternative to conventional diagnostic methods [32].

To identify new pathogenic infections in the dairy herd that can be treated with antibiotic therapy, early detection of bovine mastitis is essential. N-acetyl-β-D-glucosaminidase (NAGase) is a well-known inflammatory biomarker for bovine mastitis, which is produced in the bloodstream during pathogenesis and then released into the milk. It can distinguish between cases of subclinical and clinical bovine mastitis and healthy quarters. Adding silica-coated zinc oxide nanoparticles led to the transfer of non-radiative energy to the products of lysosomal reactions. This resulted in an improved fluorescence differentiation method to determine the severity of mastitis based on the increased fluorescence emission in a standard NAGase activity assay. This provided an efficient, straightforward and affordable way to enhance the fluorescence signal for detecting mastitis [34].

New developments in the use of nanotechnology for immunological assays

The immune-sensing system for detection of the most common mastitis- causing pathogens *staphylococcus aureus* has been proposed previously [35]. The rapid, sensitive and specific colorimetric immunological methods were developed for the identification of *Staphylococcus aureus*, a causative infectious agent of subclinical mastitis, directly from a sample of milk [36]. The development of laboratory techniques for the rapid detection and quantification of mastitis-causing pathogens, in addition to antibiotic sensitivity testing, is essential to select the most effective antibiotic against *S. aureus* mastitis [37]. Immunoassays are also utilized to detect inflammation-related biomarkers in milk at various stages of subclinical mastitis [17]. The use of nanoparticles in milk samples from subclinical bovine mastitis to detect *staphylococcus aureus* leukotoxin M/F0-PV overcomes the major disadvantages of conventional methods and can give a window for sensitive detection of toxins before the development of mastitis [38].

Enzyme-linked immunosorbent Assay (ELISA):

Many researchers are interested in ELISA because of its higher specificity, ease of use, quickness, flexibility, and relatively low cost [39]. Currently, nanomaterial-modified ELISA are more prevalent because the modification has greater potential for rapid on-site detection as it requires less reagent and can be stored at room temperature for a longer period of time [35]. The nano-ELISA kit demonstrated enhanced sensitivity and specificity, with a 93.33% sensitivity and specificity in the same samples, due to nanoparticles allowing more antibodies to enter the complex. [40]. Magnetic nanoparticles have been employed in the construction immunosorbent tests as well as in biomedical and food safety fields due to their even dispersion in solution and consistent sizes [41,42]. Through covalent immobilization, complexes between the nanoparticles and antibodies are created. The immobilized particles can be quickly separated by a magnetic field by binding with the target antigens in solution, [43,44] Moreover, the benefits of this technology include enhanced sensitivity, shorter detection times, and liquid-phase immunological reactions [45].

A new enzyme-linked immunosorbent assay that uses magnetic nanoparticles and biotin/streptavidin-HRP was developed for the quick and precise detection of zearalenone [46]. The combined utilization of antibody-conjugated magnetic nanoparticles and biotin-streptavidin system resulted in the enhancement of the detection signal and

improvement of the assay's sensitivity [47]. Furthermore, the successful application of chitosan-modified magnetite nanoparticles (CS-MNPs) as a substitute for enzymes in traditional enzyme-linked immunosorbent assay (ELISA) has been observed. CS-MNPs exhibit catalytic characteristics that facilitate the catalysis of color reactions in immunoassays, as well as magnetic properties that enable the capture, separation, and enrichment of antigens before the assay process [46]. Biotin-streptavidin coupling is one of the best characterized systems for signal amplification [48,49]. An ELISA-based diagnostic has been developed for the detection of *S. aureus*. Additionally, An anti-S. aureus monoclonal antibody-coated magnetic bead-based ELISA has been developed for the detection of Staphylococci [50].

The use of gold nanoparticles as the basis of a biomarker for protein and deoxyribonucleic acid analysis is effective due to their high absorption and optic refraction at certain wavelengths, fluorescence properties specific to optical detection techniques, with a high surface to volume ratio and unique properties [51]. DNA, antibodies, enzymes, and other biomolecules can rapidly bind with gold nanoparticles, increasing the number of biochemical detection signals [40]. The unique surface plasmon band (SPB) of gold nanoparticles (GNP) and their broad absorbance at 520 nm have drawn significant attention to GNP-based diagnostic systems [52]. The high surface area of metallic nanoparticles also contributes to their uniqueness. Furthermore, because of their large surface area bond (SPB), GNPs are also very effective quenchers over extended distances [53]. This makes them suitable for usage as quenchers for fluorescent dyes and nanoparticles.

Nanosurface energy transfer (NSET) can occur on a nanosurface in any donor orientation since there is no defined dipole movement. On the other hand, Fluorescence Resonance Energy Transfer (FRET) based immunoassays requires donor-acceptor pairs to be sufficiently close for resonance energy transfer to be activated. By comparing FRET efficiency over NSET, FRET does not give a wide range of detection and has poor detection limits. Accordingly, using the NSET phenomenon for immunoassay makes more sense [54]. Padmaja *et al*., [38] proposed a method to apply NSET phenomenon for ultrasensitive detection of LukF toxin causing bovine mastitis rather than detecting antibody Since there are several steps in the standard LukM/F′-PV ELISA, a considerable sample volume is needed to perform the test. Because of the toxicity of this method, it is crucial to detect the toxin at or below the levels that cause infection (3.6 ng/mL). Anti-rLukF polyclonal antibodies were mounted on a gold surface to create a nanoprobe that was specific to the LukF component in order to solve this problem.

Interleukin-6 (IL-6) is employed as a biomarker to assess inflammatory infections in both humans and animals and the immunofiltration assay (IFA) has been extensively utilized in the field of inflammatory biomarker identification to facilitate the sensitive and quantitative measurement of IL-6 in milk [55]. a gold-silver core-shell surface-enhanced Raman scattering (SERS) nanotag with 4-mercaptobenzoic acid 4-MBA was used for labeling in the immune filtration assay (IFA) By using an immuno-specific combination of antigen and antibody [55]. Surface-enhanced Raman scattering (SERS) has gained more interest due to a quick response time, no interference from an aqueous solution, minimal sample pretreatment needed, high accuracy, and no sample degradation [56].

Lateral flow immune-chromatographic tests:

Lateral flow assay (LFA), also referred to immune-chromatographic strip (ICS) tests, are quick tests that can reduce the waiting time for test results from hours to minutes when compared to conventional immune-chromatographic assays. These tests do not require any specialized equipment or professional knowledge for the operators. The ICS tests are appropriate for conducting on-site tests, qualitative and semi-quantitative monitoring, and to a certain extent, quantitative monitoring in settings with limited resources or lacking laboratory facilities [57]. The principle of an LFA is relies on passing a liquid sample via a polymeric strip containing molecules that interact with the substance and produce a signal that is then visualized with a nitrocellulose membrane, nanoparticles, and typically antibodies, to produce results. When a sample is added, it will flow across the test device, moving through the conjugate pad, nitrocellulose membrane, and absorbent pad [58].

Labels consist of a variety of nanoparticles (NPs) ranging from 15-800 nm in size, which enables a smooth flow through the membrane and they are frequently made of colloidal gold, latex, selenium or carbon [59]. NPs can serve as alternate labels because of their special qualities that can raise the analytical sensitivity or limit of detection of LFA [60]. Colloidal gold is currently the most popular label used in commercial LFA as the laboratory preparation of colloidal gold is inexpensive, and numerous commercial sources are available, it has a brilliant color, and no development is required for visualization, In addition, it has a high level of stability in both liquid and dry forms [58]. The immune-chromatographic strip test targeting bacterial Ribosomal protein (RP) L7/L12 protein has shown great potential as an alternative way to identify *S. aureus* in milk from cows with bovine mastitis. This test has demonstrated high sensitivity and specificity, making it a reliable and efficient tool for rapid *Staphylococcus aureus* detection from mastitis milk [59].

A semi-quantitative, sensitive, and rapid lateral flow assay targeting the myeloperoxidase enzyme of milk neutrophils was developed for early detection of subclinical mammary infection in dairy cows [25]. One of the main lysosomal enzymes found in neutrophil azurophilic granules, myeloperoxidase [25] is essential for innate defense against microbial invaders16. As a result, MPO has been extensively employed as a novel biomarker for mammary infection in cattle, and an ELISA has been created specifically for this use. Colloidal gold nanoparticles (GNP) and monoclonal anti-MPO antibodies were employed as labelling agents in a competitive immunoassay method. It had a high sensitivity as it could detect MPO at concentrations as little as 1.5 ng/ml, an accuracy of greater than 97%, and no cross-reactivity with other milk proteins. This assay could be used as a substitute for SCM diagnostic tests in laboratories able to obtain lysate of milk SCC [61]. Unfortunately, because the new test requires a somatic cell extract to be administered, it is not appropriate for usage on farms in its current state. However, if lab facilities are available to extract the lysate of milk SCC, it can be utilized as a substitute to the cow-side test of SCM detection.

A new immune-chromatographic test strip was created utilizing gold nanoparticles-*staphylococcus aureus* monoclonal antibody conjugates for the quick and easy identification of *staphylococcus aureus*, throughout a double-antibody sandwich format, showing significant potential due to its fast, low-cost, and favorable sensitivity and specificity, when compared with traditional techniques for bacterial detection [62]. This test strip can detect 98.7 % of bacteria at a detection limit of 103 CFU/ml. within 10 minutes, the results are visible to the naked eye, making it a viable option for the quick detection of *staphylococcus aureus* in food. This immunochromatographic assay-based test strip has great potential for use in food safety control systems and clinical diagnosis because it is simple, fast, cheap, sensitive, and specific compared to traditional bacterial detection methods [62]. Recent advancements and future targets for improving LFAs include developing new signal amplification methodologies and nanoparticle labels [58].

New developments in the use of nanotechnology for molecular assays:

Molecular diagnostic tools are increasingly used in mastitis diagnosis as they enable rapid, qualitative, quantitative and large-scale diagnosis (El-Sayed et al., 2017). Significantly, nanotechnology improves molecular diagnosis towards the nanoscale and several nanoparticles including gold nanoparticles and magnetic nanoparticles have several applications in molecular diagnostics [63]. LAMP test have been reported for detecting mastitis-causing microorganisms such as *S. aureus*, *S. agalactiae, and S. uberis* from bovine mastitis milk samples [64]. Various studies are seeking to improve a LAMP assay that can identify mastitis-causing bacteria in bovine mastitic milk, specifically *Staphylococcus aureus*, *S. agalactiae*, and *Streptococcus uberis* from [65]. Moreover, PCR is the most widely used technique in modern molecular biology study. Generally, it is a technique used to increase the number of copies of a specific DNA sequence [66].

Current molecular methods are constantly being refined, and new methods are being created to reduce costs while offering increased sensitivity and specificity [67]. In molecular diagnostics, nanotechnology—the production and use of materials, tools, and systems by manipulating matter at the nanoscale—has been utilized. Moreover, some of the nanotechnologies that allow for single-cell and molecule-level diagnosis can be added to existing molecular diagnostics to further expand their capabilities [19].

Polymerase chain reaction (PCR)

The application of nanoparticles in conventional PCR improves the procedure as nanoparticle (NP)-assisted PCR can significantly reduce the total process time and process specificity. This is essential for the technique's application in medical diagnosis and infield detection [68]. In addition, NP- enhanced PCR has higher efficiency due to less required expensive reagents which significantly reduces the cost of experiments [69]. One of the significant metal nanoparticles used in several study domains is iron nanoparticle (MNP) [70,71]. In the domains of biology and medicine, modified magnetic nanoparticles (MNPs) are widely used in target cell separation, DNA extraction, and purification. By forming electrostatic, hydrogen, and covalent bonds, the amine form nanoparticles might be utilized for protein and pathogenic bacterial separation and extraction [72,73]. Additionally, bacteria, fungi, and viruses are targeted and extracted using magnetic nanoparticles modified with tiny biomolecules including cytosine, mannose, antibodies, Aptamers, DNA biosensors, and antibiotics. The design and selection of suitable functional groups can improve the efficiency and specificity of the nanoparticles [74].

To improve the selectivity of detecting S. aureus target DNA, DNA biosensors were coupled with MNPs. Target DNA was extracted by a magnet and released by release buffer after bonding to the nano-biosensor. 260 nm spectrophotometry measured target DNA absorption and concentration. The developed nano-biosensor was more sensitive than conventional PCR and electrophoresis techniques at different bacterial concentrations and completely selective for *S. aureus* target DNA [74].

The rapid identification of *Staphylococcus aureus* in milk samples without the need for culture enrichment was achieved through the combination of

lateral flow recombinase polymerase amplification (RPA-LF) with immune-magnetic separation (IMS), with a detection time of 70 minutes. The method involved DNA amplification using RPA at 39°C for 10 minutes, followed by visualization of the amplicons through LF strips for 5 minutes, resulting in the detection of S. aureus within 15 minutes. This approach is superior in terms of sensitivity and specificity compared to the conventional polymerase chain reaction [75].

Loop-mediated isothermal amplification (LAMP):

LAMP is a type of sequence-specific isothermal amplification technique that provides nucleic acid amplification at a fixed temperature (typically 60–67ºC) using 4 to 6 primers and a polymerase with chain displacement activity [76]. The advantages of LAMP for clinical diagnosis are its high sensitivity and specificity, speed (less than an hour), ability to amplify at different pH and temperature ranges, and the stability and low cost of its reagents [77].LAMP has been applied in a variety of different fields, including molecular diagnostics [76]. The analysis of LAMP products has been carried out using several techniques such as agarose gel electrophoresis, visual observation of color changes, and turbidity [78]. However, these methods are not specific to the target genes and may produce incorrect positive results. To address this issue, a simple and visually observable nanoparticle-based lateral flow biosensor (LFB) detection method has been developed, which is specific to the target genes. This method has been successfully used for the analysis of m-LAMP products [79].

LAMP test have been reported for detecting mastitis-causing microorganisms such as *S. aureus*, *S. agalactiae, and S. uberis* from bovine mastitis milk samples[64].Various studies are seeking to improve a LAMP assay that can identify mastitis-causing bacteria in bovine mastitic milk, specifically *Staphylococcus aureus*, *S. agalactiae*, and *Streptococcus uberis* from bovine mastitic milk [65]. The best diagnostic results were found with m-LAMP-LFB, which refers to multiplex loop-mediated isothermal amplification linked to a nanoparticle-based lateral flow biosensor. Both sensitivity and specificity reached 100%. FLB is used to analyze LAMP diagnosis results [80]. Multiplex loop-mediated isothermal amplification combined with nanoparticles-based lateral flow biosensor (m-LAMP-LFB) is a new diagnostic method that is fast, reliable, and simple to use to identify S. aureus and MRSA from methicillin-susceptible S. aureus (MSSA) [81].

Conclusion

Enhancing the diagnostics for subclinical bovine mastitis is crucial to facilitate precise and timely identification of *staphylococcal aureus*. In addition, this reduces financial losses and protects public health by preventing mastitis and improving dairy animal care. Recently, nanotechnologies have been employed successfully to enhance current colorimetric, immunological and molecular diagnosis methods of bovine subclinical mastitis. The reason for materials at nanoscale to show enhanced properties from their conventional counterparts is owing to two effects; quantum confinement effect and larger surface-to-volume ratio. Nanotechnology made acceleration to the field of diagnosis of mastitis suggests that, in the future, research should be directed toward construction of nano-structured platforms for ultrasensitive identification of subclinical mastitis causing toxins and pathogens.

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Authors' contributions

Research work was done according to the study protocol.

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تشخيص التهاب الضرع البقري الكامن الناتج من ميكروبات المكورات العنقودية الذهبية باستخدام تقنيات النانو

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المستخلص

يعد التهاب الضرع أحد أكثر الأمراض المعدية شيوعًا والتي تسبب معظم الخسائر المالية لمزارع الألبان. الخطوة الأساسية بين تحديد مسببات المرض وإيجاد العلاج هي التشخيص الدقيق. يبذل الباحثون جهدًا لتطوير طرق تشخيص حساسة ومحددة وسريعة وفعالة من حيث التكلفة لالتهاب الضرع البقري الكامن الناتج عن المكورات العنقودية الذهبية، وهناك حاجة ماسة لحل المشكلات المتعلقة بطرق التشخيص الحالية. توجد حاليًا تطورات جديدة في استخدام التقنيات القائمة على تكنولوجيا النانو للكشف اللوني والمناعي والجزيئي، ويتم بذل المزيد من الجهود لتصميم أجهزة استشعار حيوية للكشف الدقيق والانتقائي والوظيفي عن الجزيئات الحيوية. تعتبر الجسيمات النانوية جيدة للاستبدال والدمج مع طرق تشخيص التهاب الضرع الكامن التقليدية الأخرى نظرًا لحجم الجسيمات النانوية وميزاتها الفيزيائية والكيميائية القابلة للتكيف بما في ذلك؛ الخصائص الكهربائية والكهروكيميائية والبصرية والمغناطيسية. ومن ثم، فإن هذا الاستعراض يناقش تطبيقات ودور تكنولوجيا النانو في تشخيص التهاب الضرع البقري الكامن الناتج عن عدوي المكورات العنقودية الذهبية

الكلمات المفتاحية: التهاب الضرع الكامن، التشخيص، تقنية النانو، التحاليل المناعية، التحاليل الجزيئية، التحاليل ذات النتائج الملونه.