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Recent progress in the genotyping of bovine tuberculosis and its rapid diagnosis via nanoparticle-based electrochemical biosensors

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Bovine tuberculosis (bTB) is considered a worldwide infectious zoonotic disease. *Mycobacterium bovis* causes bTB disease. It is one of the *Mycobacterium tuberculosis* complex (MTBC) members. MTBC is a clonal complex of close relatives with approximately 99.95% similarity. *M. bovis* is a spillover pathogen that can transmit from animals to humans and rarely from humans to animals with contact. Genotyping techniques are important to discriminate and differentiate between MTBC species. Spoligotyping and mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) are widely used but they have some limitations. As an alternative, whole genome sequencing approaches have been utilized due to their high-resolution power. They are employed in typing *M. bovis* and explain the evolutionary and phylogenetic relationships between isolates. The control of bTB disease has attracted a large amount of attention. Rapid and proper diagnosis is necessary for monitoring the disease as an initial step for its control and treatment. Nanotechnology has a potential impact on the rapid diagnosis and treatment of bTB through the use of nanocarrier and metal nanoparticles (NPs). Special attention has been paid to voltammetric and impedimetric electrochemical strategies as facile, sensitive, and selective methods for the efficient detection of tuberculosis. The efficacy of these sensors is enhanced in the presence of NPs, which act as recognition and/or redox probes. Gold, silver, copper, cobalt, graphene, and magnetic NPs, as well as polypyrrole nanowires and multiwalled carbon nanotubes have been employed for detecting tuberculosis. Overall, NP-based electrochemical sensors represent a promising tool for the diagnosis of bTB.

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Introduction

Tuberculosis (TB) is an important zoonotic disease of animals and humans.¹ Despite advancements in its prevention and management, TB still ranks as one of the top infectious agents in terms of mortality and has a devastating impact on populations of bovine livestock and wildlife worldwide.² A World Health Organization (WHO) study from 2022 reported new cases of nearly ten million and 1.6 million fatalities as a result of initial infection, reinfection, or re-activation of latent infection (*Tuberculosis Deaths and Disease Increase during the COVID-19 Pandemic*, n.d.), and the increasing incidence of antimicrobial resistance (AMR) is a hazard to public health.³ WHO indicated

that there are eight countries showing two-thirds of the novel cases: China, Nigeria, India, Bangladesh, Indonesia, South Africa, Pakistan, and the Philippines. Additionally, TB incidence was declining globally by an accumulated decreasing rate of 11%, which is less than half of the anticipated 20% drop from 2015 to 2020 outlined in the previous TB policy. Eradicating tuberculosis by 2030 is one of the first health goals of the United Nations Sustainable Development Goals (SDGs) (*Tuberculosis*, n.d.). Furthermore, bovine TB (bTB) is notifiable disease that must be informed to the OIE (World Organization for Animal Health). Reporting of the bTB status from 188 different countries from January 2017 to June 2018 revealed that the presence of the disease in animals was 44% with prevalence increasing in Africa, America and some regions of Asia.⁴ Regardless of successful worldwide notification calls, the real effect of bTB in livestock is rarely adequately reported, particularly in wild animals and in nations with undeveloped control measures.⁵

bTB is caused by *M. bovis* and it is considered to spill-over between hosts.⁶ It usually spreads to people after close contact with diseased animals or the consumption of soiled, unpasteurized dairy products.⁷ The symptoms of the human disease of *M. bovis*, which is resistant to PZA,⁸ one of the first TB

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medications, are like those caused by *M. tuberculosis*.⁹ It has been assessed that *M. bovis* accounts for less than 1.5% of all human cases of TB in areas outside of Africa and almost 2.8% of all TB disease in Africa.¹⁰

Effective programs to eradicate and control bTB usually depend on testing and slaughtering, post mortem inspection actions and movement restrictions, and before that proper diagnosis.¹¹ To understand TB transmission, bacterial phylogeny and evolution has been considerably enhanced with the invention and application of molecular genotyping approaches for *Mycobacterium* species. Genotyping of *Mycobacterium tuberculosis* complex (MTBC) species is considered common practice for epidemiological tracking, and the analysis of regional and global strain population dynamics are of significant importance to investigate the spread of extensively drug-resistant (XDR) and multidrug-resistant (MDR) bacteria.¹² Several methods for conventional genotyping can be used to examine several classes of genetic markers and produce strain-specific banding patterns (IS6110 DNA fingerprint), bar code-like signals (spoligotyping), or numerical patterns (24 locus-MIRU-VNTR typing).¹³ Nevertheless, quick advancements in whole-genome sequencing (WGS) methods offer more thorough insights into the underlying molecular and epidemiological techniques and result in improved diagnostic and treatment tools.¹⁴

In the past, *M. bovis* genotyping methods have mostly relied on PCR-based assays to evaluate a few of the genetic markers.¹⁵ Whole genome sequencing (WGS) of *M. bovis* will eventually substitute some challenging analyses as the cost per genome continues to drop, while also allowing for the required high resolution analysis of outbreaks.¹⁶ These genetic techniques offer valuable insights into the causes of infection, the transmission routes, the geographic localization, and the preferred hosts, as well as the dynamics and risk factors of the disease,¹⁷ thus promoting the reduction or elimination of the infection.^{18,19} Hence, this review will illustrate different genotyping methods, and WGS application in the detection of genetic diversity, and tackle the dynamic transmission of the *M. bovis* pathogen. On the other hand, the diagnosis of tuberculosis is necessary as a preliminary step for its control and treatment. In this review, a great focus has been put on electrochemical sensors as a rapid tool for the diagnosis of tuberculosis.

Electrochemical sensors represent an effective and rapid tool for determining various organic, inorganic, and biological molecules, as well as microorganisms.^{20–22} Electrochemical sensors can be classified into various types such as potentiometric, voltammetric, impedimetric, amperometric, and electrogenerated chemiluminescence sensors.^{20,23} The sensing process relies on the redox behavior of the targeted analytes at surfaces of the electrodes. For superior sensitivity and selectivity, as well as lower detection limits, the electrodes are modified with electroactive materials such as polymers, ferrocenes, and nanoparticles (NPs).²⁰ Herein, a great focus has been put on NPs, such as gold (Au), silver (Ag), copper (Cu), cobalt (Co), ferric oxide (Fe₃O₄), graphene, poly pyrrole (PPY), and multiwalled carbon nanotubes (MWCNTs), which have been used for the electrochemical detection of tuberculosis.

Additionally, NPs as nanocarriers for the delivery of antituberculosis drugs are discussed.

A concise summary of MTBC genomics

For the accurate interpretation of genotyping and WGS data, understanding the genetic profile of *M. bovis* is required. It is involved in the *Mycobacterium tuberculosis* complex (MTBC), a group containing 11 species of bacteria with variation in virulence and host tropism.²⁴ MTBC genomes are very similar with higher than 99.95% homology at the level of nucleotides. These genomes contain ribosomal RNA genes (rRNA), while horizontal gene transfer (HGT) and significant recombining actions are thought to be missing.²⁵ The only evolution occurring in the *Mycobacterium* pathogen can be due to indels (small insertions and deletions), single nucleotide polymorphisms (SNPs), deletions of about 26 kbp, duplication of a few paralogous genes and shuffling of insertion sequences (IS).^{26–28} Differential hybridization arrays and physical mapping have been carried out on *M. tuberculosis* H37Rv, *M. bovis* ATCC 19210 and *M. bovis* BCG Pasteur strains. Fourteen evolutionarily large deletion regions known as “regions of difference” (RD1–14) were characterized that had different sizes and presence in the strains.^{29,30} Identification of RDs is regarded as the gold standard technique to distinguish between MTBC species.³¹ Consequently, the deletion of RD4 and RD9 of *M. bovis* and RD1 from *M. bovis* BCG strains were used in the precise differentiation from other MTBC members.³²

The *M. bovis* genome is about 4.3 Mb in size, covering around 4216 genes, with one copy of (5S, 16S, and 23S) rRNA genes, and 45 tRNAs with high GC content representing 65%, which infers proper sequencing for WGS library creation.³³ One of the biggest difficulties in analyzing WGS data is the high proportion of repeated elements in MTBC genomes, including those of *M. bovis*. These involve IS, integrases, two phage sequences, clustered regularly interspaced short palindromic repeats (CRISPR), proline proline glutamate (PPE) or proline glutamate (PE) family genes, and genes from the 13×10^{12} repeat family. In particular, the family gene of PE–PPE is reported in about 10% of MTBC genomes, and has been linked with TB pathogenicity.^{34,35} As most widely used sequencing machines produce reads shorter than repeats, repetitive region handling becomes a challenge in genomic investigations. Traditional genotyping methods have been developed over time and are based in part on some of these repetitive regions.³⁶ Table 1 shows the characteristics of genotyping and WGS.

Conventional genotyping techniques of *M. bovis*

The conventional genotyping techniques of *M. bovis* intervention include restriction endonuclease analysis and pulsed-field gel electrophoresis, IS6110-RFLP, PGRS-RFLP, spoligotyping, variable number tandem repeat (VNTR), and multispacer sequence typing (Fig. 1).





Table 1 Different characteristics of genotyping techniques and WGS

Method	Principle	Minimum DNA input	Time	Advantages	Disadvantages	Ref
REA	Using three different restriction enzymes (PvuII, BstEI, and BclI) in total DNA of <i>M. bovis</i>	High (4–6 µg)	2 days	- Applied for strain typing in <i>M. bovis</i> , - Standardized techniques	- Too many bands that are hard to resolve - Needs <i>M. bovis</i> isolation - Technically challenging	37 and 79
PFGFGE	Digestion DNA with rare cutting enzymes and the use of agarose gel to separate by electrical pulses	High (2 µg)	7 days	- Able to differentiate between MTBC species	- Labor intensive - Low intra-specific discrimination - Intact DNA is needed for restriction enzyme treatment - It is complicated getting agarose plugs - Requires isolation of <i>M. bovis</i> - Not defined standard method - Long turn-over time - No discriminatory power for <i>M. bovis</i> - Labor intensive - Technically demanding - Requires <i>M. bovis</i> isolation	40 and 41
IS6110-RFLP	Restriction enzymes digest <i>M. bovis</i> whole DNA, then electrophoresis on agarose gel, and southern blot with IS6110 probes	High (2–3 µg)	3 to 4 days	- Standardized methods - Specified for <i>M. tuberculosis</i> typing, but not <i>M. bovis</i>	- Standardized methods - Labor intensive	54 and 55
PGRS-RFLP	Restriction enzymes digest <i>M. bovis</i> in whole DNA, then electrophoresis on agarose gel, and southern blot with PGRS probes	High (2–3 µg)	3 to 4 days	- Standardized methods - Used in typing <i>M. bovis</i>	- Too many bands that are hard to resolve - Labor intensive - Technically demanding	57
Spoligotyping	PCR dependent method detecting the existence of 43 distinctive short DNA sequences (or spacers) in the DR position	Very low (10 fg) (even from extracted infected tissue)	2 to 3 days (up to 40 samples)	- Standardized procedures - Extracted DNA can be directly used - Automatization - High throughput - Used in typing <i>M. bovis</i> - Standardized procedures - Extracted DNA can be directly used	- Homoplasy - Lower resolution power compared to REA and MIRU-VNTR - Labor intensive	59 and 69
MIRU-VNTR PCR	PCR-based amplification of VNTR loci and visualization following gel electrophoresis, MIRU-VNTR PCR targets many genomic loci	Very low (10 fg) (even from extracted infected tissue)	1 to 2 days for a few samples ^a	- Standardized procedures - Extracted DNA can be directly used - Automatization - High throughput - Used in typing <i>M. bovis</i> - Widely use in <i>M. tuberculosis</i> - Sequencing and accessibility of analyzed sequences in public databases - Infers evolutionary relationships and correlate geographical location	- Variable discriminating power depending on the chosen loci - Homoplasy	59 and 69
MST	Based on an examination of many intergenic regions from a single sequence	2 µL target DNA	Fast		- Limited in <i>M. bovis</i>	78 and 80

Table 1 (Contd.)

Method	Principle	Minimum DNA input	Time	Advantages	Disadvantages	Ref
WGS	DNA is digested or sheared, and all fragments are simultaneously sequenced	Low to high ^b (1 ng to 1 µg)	2 to 4 days ^c (~24 to 96 samples)	- Standardized techniques for sequencing - High output - Reproducible - High resolution - Detects spoligotypes and MIRU-VNTR patterns - Used in typing <i>M. bovis</i> - Used for evolutionary research of <i>M. bovis</i>	- Requires <i>M. bovis</i> isolation - Repeats are hard to resolve using short reads - Technically demanding - Needs more advanced bioinformatic skills - Data analysis is needed	70

^a Time can also vary depending on the number of tested samples. ^b Total DNA input for WGS depends on the chosen library. ^c Run time is highly dependent on the chosen protocol.

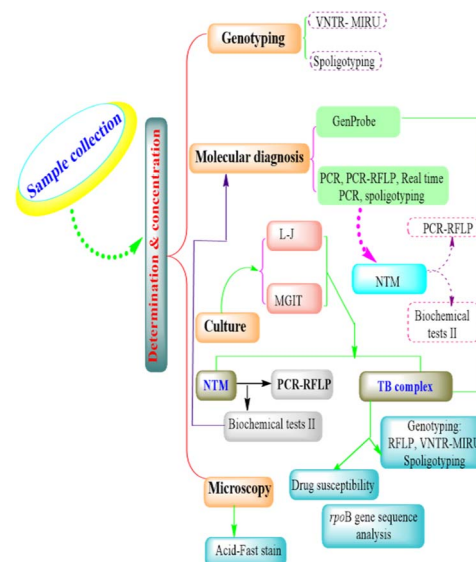


Fig. 1 Flow chart showing conventional genotyping techniques of *M. bovis* intervention.

Restriction endonuclease analysis and pulsed-field gel electrophoresis

The initial approach to *M. bovis* intraspecific typing, restriction endonuclease analysis (REA), involves the rapid digestion of large quantities of whole DNA from *M. bovis* samples using three separate enzymes (PvuII, BstEII, and BclI), followed by the visualization of band patterns on agarose gels.³⁷ In spite of its use in molecular epidemiology investigations, it seems to have significant technical drawbacks because it utilizes too many little, difficult-to-reconstruct DNA fragments.³⁸ Recently, it has been no longer used other than as a reference in New Zealand, where it was created, and it was last employed for normal *M. bovis* typing in 2011.³⁹

The third-generation molecular technique for bacteria typing, known as pulsed-field gel electrophoresis (PFGE), eventually emerged for *M. tuberculosis* as well as the other MTBC species. Its resolution patterns were better than REA (fewer and bigger bands).⁴⁰ Nonetheless, PFGE had two major drawbacks: (1) the lipid rich cell wall of the mycobacterium hinders the PFGE's lytic enzymes from working as intended, impairing the utilization of the agarose plugs;⁴¹ (2) comparative investigations revealed that the PFGE of *M. tuberculosis* strains had a low discrimination power *versus* later developed genotyping methods. PFGE has certain inherent disadvantages, like the fact that it is time consuming and labor intensive.^{42,43}

Considering the failure of REA and PFGE to distinguish between *M. bovis* and *M. tuberculosis* strains, seeking polymorphic and stable genetic markers permitted the validation of more accurate genotyping methods. Now, there are genetic markers applied commonly including the direct repeat (DR) region, IS6110 (for *M. tuberculosis*), the variable number tandem repeats (VNTR) sequences and the poly (GC) rich sequences (PGRS). Each marker has its typing style that goes with it.

IS6110-RFLP

IS6110 is a particular insertion sequence (1358 bp) of the MTBC, and variations in its position and number of copies are what differentiate between isolates.⁴⁴ This insertion sequence can serve like a mobile promoter, induce structural variation (insertion, deletion or inversion), and encourage gene silencing.^{45,46} In a case in Spain, *M. bovis* initiated a significant multidrug resistant tuberculosis (MDR TB) outbreak in humans linked to gene overexpression and increased virulence by IS6110 in *phoP* promoter.⁴⁷ Moreover, the association between the number of copies of the *M. bovis* strain and the high prevalence of bTB resulted from genomic alterations driven from IS6110 transposition.⁴⁸ Also, the high IS6110 content in the *M. tuberculosis* Beijing lineage enhanced its virulence, antibiotic resistance, and environmental adaptability.^{49,50}

For identifying IS6110 in the *M. tuberculosis* strain, the standard frequently utilized approach is IS6110-RFLP (IS6110-restriction fragment length polymorphism).⁵¹ Briefly, the approach entails high concentrations (2–3 g) of entire bacterial DNA extraction, *Pvu*II endonuclease digestion, and standard agarose gel electrophoresis. Then, the gel is applied to complete a Southern blotting, as the fragments of DNA have been moved to a membrane and probes complementary to the 3' end prime of the IS6110 sequence. This is followed by hybridization to disclose the size of the generated fragments and the number of IS elements using chemiluminescence (originally radiolabeling).^{52,53}

A main disadvantage of the IS6110-RFLP method is that the *M. bovis* strain has a single or few copies of the insertion element⁵⁴ and this affects the discrimination power of this approach due to the same IS6110-RFLP pattern.⁵⁵ Furthermore, this technique is labor intensive and needs large amounts of DNA, just like REA and PFGE. For these reasons, despite being often utilized for *M. TB*, *M. bovis* genotyping does not benefit much from IS6110-RFLP.

PGRS-RFLP

GC content is a valuable parameter for classification and differentiation of MTBC members.⁵⁶ Polymorphic GC repeat sequence (PGRS) is performed to improve the power of identification of IS6110 isolates for *M. bovis*, but this also demands large DNA quantities and highly technical skills.⁵⁷

Spoligotyping

Spoligotyping is a rapid PCR dependent technique that detects the existence of 43 distinctive short DNA sequences (or spacers) in the DR position.⁵⁸ This approach relies on amplification of the DR, followed by hybridization of the amplified products with membrane bound oligonucleotides that are related to the variable spacer areas located between the DRs.⁵⁹ The hybridization signals are recognized by chemiluminescence *via* biotin labeling of the PCR products and a streptavidin–peroxidase conjugate system and then observed using autoradiography.⁶⁰ Fig. 2 illustrates tuberculosis (TB) spoligotyping intervention.

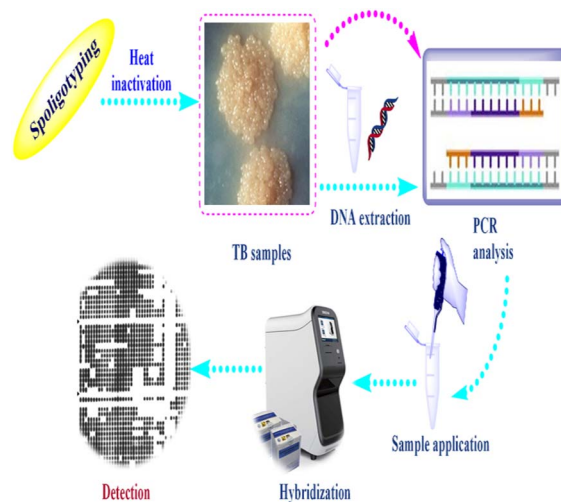


Fig. 2 Flow chart showing tuberculosis (TB) spoligotyping intervention.

Patterns of spoligotypes are defined by their identity and the number of spacers. These patterns can be used to distinguish between similar or dissimilar strains. The lack of (3, 9, 16, and 39–43) spacers in *M. bovis* help in species distinction.⁶¹ This method has the benefit of being able to use DNA isolated from infected tissue samples without the need for bacterial isolation.⁶²

Further automatization and development of this method has been directed to employ recognition systems based on microbeads, like Luminex platforms,⁶³ multiplexed primer extension depending on spoligotyping assay using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS),⁶⁴ microarray,^{65,66} melting curve analysis and ligation based amplification.⁵⁸ One of the most important advantages of spoligotyping is its ability to be used directly in clinical samples without the requirement for preparatory culture due to its true sensitivity estimated at 10 fg of chromosomal DNA, or the DNA of 2–3 bacterial cells.⁶⁷ Also, it has shown promise in the characterization of nonviable cultures, Ziehl–Neelsen smear slides, and paraffin-embedded tissue sections.⁶⁸ The drawback of spoligotyping is homoplasy as irrelevant lineages might exhibit similar patterns due to lack of sequences of spacers, which makes spoligotypes poor predictors of phylogenetic relatedness.⁶⁹ In addition, it has limitations in the detection of the spread of the disease on the farm scale because of the poor resolution.⁷⁰ Despite these considerations, spoligotyping is still the best practical genotyping method in *M. bovis* research.

Variable number tandem repeat (VNTR)

An arrangement of tandem repeats that are orientated in the same direction and clustered together is known as a VNTR locus. The locus's size (measured in base pairs) fluctuates depending on how often the nucleotide sequence is repeated. Recombination or replication defects allow for the addition or deletion of each repeat, resulting in alleles with various repeat



counts.⁷¹ In contrast to the one DR site used for spoligotyping, there are numerous VNTR loci in MTBC that can be found using PCR. The count of repeats in every position is reflected by the size of the PCR products that are produced. There are several types of VNTR, including MIRU, Mtu and QUB.^{72,73}

MIRU-VNTR needs small amounts of non-purified DNA and produces results in a digital format that may be transferred and used to build national and global databases for routine and research use.⁷⁴ It makes use of repeated sequence changes that are not subject to selection pressure and evolve quite quickly, making them appropriate for future molecular epidemiological investigations and surveillance.⁷⁵ A 24-loci MIRU-VNTR PCR is frequently employed, and it is reported to have poor accuracy in distinguishing between samples of *M. tuberculosis*, but this is not proven for *M. bovis*.⁷⁶ The MIRU-VNTR approach provides a greater level of discrimination than spoligotyping, is quicker than RFLP analysis, and involves simple interpretation, as well as being familiar to technicians.⁷⁷ MIRU-VNTR is also affected by homoplasy⁶⁹ and assessment of the 24 loci of *M. bovis* for preventing homoplasy is time consuming and laborious.⁷⁰

Multispacer sequence typing

Multispacer sequence typing (MST) is a sequencing dependent approach which can help in the epidemiological research of *M. bovis* by linking several polymorphic markers and utilizing intergenic regions sensitive to high mutation levels given the weak selection power. MST is employed for the *M. tuberculosis* strain, and not for the *M. bovis* strain. In the first report, Sales *et al.* identified the variability of *M. bovis* isolates using the MST approach in the short term. They also accompanied MST with the spoligotyping method, and the findings strengthen MST as a substitute technique for *M. bovis* genotyping. It has the benefits of sequencing and accessibility of analyzed sequences in public databases, inference of evolutionary relationships, and usage as a tool for further study by specialists worldwide.⁷⁸

Risk factors and prevalence of *M. bovis* in Egypt

Kasir *et al.* revealed that *M. bovis* has been reported in animals and humans in only eight nations in the Middle East and North Africa (MENA): Egypt, Algeria, Iran, Iraq, Sudan, Morocco, Tunisia and Turkey. In other MENA nations, Djibouti, Lebanon, Saudi Arabia and Palestine, only human cases of zoonotic TB (zTB) have been recorded.⁸¹ The variation of *M. bovis* prevalence is influenced by the population size and characteristics, geographical region, and applied diagnostic and examination schemes. In addition, bTB prevalence is also linked to other factors, like BCG vaccination status, contaminated dairy product consumption, and the effectiveness of bTB control measures.⁸² The prevalence of *M. bovis* seems to be significantly influenced by a variety of bTB risk factors. Age, sex, animal body condition, crowding, grazing practices, cross-species transmission, immunosuppression, environmental conditions, feeding systems, and pathological and physiological differences are powerful parameters affecting the spread *M. bovis*. Because

of parturition, gestation and lactation, female animals are more susceptible to bTB than male animals.^{16,83} Cross species transmission between (cattle and buffalo) and (cattle and goats) has been correlated to grazing locations and shared drinking water in Iran and Algeria, respectively.^{84,85} Shared water transport has great zoonotic importance for the transmission of infectious diseases and acquired AMR.^{86,87} Furthermore, unregulated animal migrations and international trade have been cited as major factors contributing to the spread of bTB.⁸⁸ Workers (*e.g.*, veterinarians, farmers, slaughterhouse workers) who had a close relationship with livestock, especially dairy cattle, or who dealt with wild animals, were more vulnerable to *M. bovis* infection.⁸⁹ Cattle and buffalo were the two animal species in which active bTB was most frequently recorded; rare instances among other species were also noted. In Egypt, *M. bovis* was recorded in a mongoose,⁹⁰ camels and pigs.⁹¹ Numerous research revealed that the prevalence in Egypt was between 0.1%⁹² and 16.4%.⁹³ Furthermore, studies with limited population sizes revealed a greater incidence, ranging from 22.2% (ref. 94) to 82.6% (ref. 95) in Egypt. Over time, bTB prevalence patterns have changed; in Egypt, they have ranged between 0.2% (ref. 96) and 4.3%.⁹⁷ Moreover, the majority of cases of bTB in both humans and animals have been found in the lungs and extrapulmonary regions.⁸¹ In humans, an investigation demonstrated that out of 45 pulmonary and 67 extrapulmonary tuberculosis cases, only one was a result of *M. bovis* in Egypt.^{98,99} Additionally, when directed at occupational risk groups, accessible data revealed high percentages of zTB, reaching 5.36% in Egypt.¹⁰⁰

Whole genome sequencing (WGS) process

The process of WGS begins with DNA extraction and purification. There are different generations of sequencing technologies; second-generation sequencing technologies, such as Illumina, produce short reads on the order of 50–200 base pairs and have low error rates of around 0.5–2%, with the errors chiefly being substitution errors; third-generation technologies, such as PacBio, and fourth-generation technologies, such as Oxford Nanopore, provide read lengths in the thousands or tens of thousands but have much higher error rates of around 10–20%, with errors being chiefly insertions and deletions.¹⁰¹ Next, the ends are blunted and joined to oligonucleotides known as adapters. Usually, PCR is used to amplify these fragments before the final DNA library is applied to the sequencer. Two readings are produced for each genome after the run is complete. These readings must be put together; this process aims to overlap reads to create a segment of the DNA sequenced.¹⁰² The result file, which contains a number of contigs (DNA sequences) that the assembler was unable to shut, will be created after the assembling process is complete. Depending on the library preparation, genomic nature, assembly workflow, *etc.*, the number of contigs per sample varies not only between different samples but also between the same sample and different runs. As a result, they are typically utilized to create the



genome reference, followed by resequencing on one of the previous mentioned platforms (such as Illumina). The enormous amount of output data generated requires specialist bioinformaticians to perform proper analysis.¹⁰³

WGS insights into the dynamic transmission of *M. bovis*

The dynamic transmission of *M. bovis* has various modes, which need an ecosystem level approach. In this approach, the environment, people, and animals within society must all be taken into account.¹⁰⁴ Since the pathogen can be excreted by a variety of routes—including vaginal secretions, milk, feces, urine, and/or semen—age, hygiene practices, and environment all have an impact on the cattle's infection routes.¹⁰⁵ Interspecies transmissions have occurred in nations where *M. bovis* is known to exist in wildlife.¹⁰⁶ The wildlife reservoir hosts of *M. bovis* include the European badger in Ireland and Great Britain, the brush-tailed possum in New Zealand, African buffalo in South Africa, white-tailed deer in Michigan, USA, wild boar in Peninsula,¹⁰⁷ and wild boar, goats,¹⁰⁸ fallow deer, red deer, and roe deer in Europe.¹⁰⁹

Despite some challenges of whole genome sequencing (WGS) in low and middle income countries,¹¹⁰ it has great impact on the discrimination and clarification of the entire DNA sequence. It allows a great degree of resolution, and comprehensive genetic insights including all potential genomic targets, transmission, genetic evolutionary, virulence determining factors, and drug resistance character.¹¹¹ A molecular typing and WGS investigation was conducted in Egypt's Nile Delta to address the dynamic transmission of *M. bovis* among dairy cattle. The results found that a prominent spoligotype (SB0268) emerged between 2013 and 2015, signifying a current clonal expansion of this isolate inside the Nile Delta. Remarkably, two isolates belonged to the *M. bovis* BCG group, although Egypt does not permit animal vaccination. The remaining isolates belonged to the dangerous *M. bovis* clonal complex European 2, which is present in various European and Latin American nations.¹¹² Furthermore, in Turkey, a clonal similarity study was carried out between cattle and human isolates using the spoligotyping and MIRU-VNTR techniques. The positive results hinted that *M. bovis* may be correlated to lung tuberculosis in humans.¹¹³ In Brazil, 11 spoligotype patterns were detected from 40 (25%) isolates of *M. bovis*, with a predominance of SB0121 (25.0%), SB1142 (37.5%), and SB1145 (10.0%). Other patterns, SB1050, SB0295, SB1144, SB1802, SB0881, SB0120, SB0140, and SB0849, with different values from 2.5 to 7.5%, were unevenly disseminated throughout the cities and provided epidemiological evidence of their potential exchange.¹¹⁴

M. bovis diversity values are highest in Northern and Eastern Africa, followed by Central Africa, and are lowest in Southern and Western Africa. It has been proposed that *M. bovis* originated in the Near East, travelled throughout Africa with domesticated animals, and resulted in the acquired ability to digest milk biologically as an adult.¹¹⁵ Moreover, a study carried

out in Ethiopia (September 2018 to June 2019) showed that bTB is caused by *M. bovis* SB0033 and SB1176 strains with weak recurrence.¹¹⁶

A systematic assessment of 15 research studies revealed that *M. bovis* was the causative agent of all verified instances of zTB in humans in the range 0% to 28%. The predominant exposures to zTB were from cattle and raw dairy. Eight of the 15 studies sampled livestock, mostly cattle, and found that zTB prevalence ranged from 0% to 23%.¹¹⁷ Another meta-analysis review was performed among humans to estimate the *M. bovis* prevalence from 20 April 2009 to 17 April 2019. It showed that human *M. bovis* cases occurred globally, and this told us that zTB remains important in all regions. Methodological discrepancies and the requirement for additional molecular studies make it challenging to comprehend the full picture of illness prevalence.¹¹⁸ Kwaghe and his collaborators recommended necessary set up control measures to eliminate TB in cattle and humans in Nigeria. SB1025 and SB0944 were revealed as the patterns of *M. bovis* strains in cattle, while Haarlem families and LAM 10 were related to *M. tuberculosis* strains in humans.^{119,120} However, in Ghana, Acquah *et al.*'s research revealed that about 29% of *M. bovis* strains causing bTB are uncharacterized spoligotypes, so more control is needed for bTB in Ghana.¹²¹ Sandoval-Azuara *et al.* performed WGS and SNPs for cattle and human *M. bovis* isolates. They found that all isolates from humans and cattle were matched in the spoligotype patterns, and all human isolates shared common ancestors with cattle in Baja California. This implies that most tuberculosis reports in humans caused by *M. bovis* are obtained from travelling cattle.¹²²

Applying WGS for *M. bovis* isolates from populations of elk, deer, and cattle revealed that bTB in elk and cattle gathered in the same clade, and this implies either intra-species transmission, or the sharing of a common source.¹²³ In South Africa, goats are considered as a potential source of *M. bovis* for humans, cattle, and wildlife.¹²⁴ Another novel record of bTB transmission is between cattle populations and badgers in Great Britain.¹²⁵ Lombard *et al.* proved that MTBC could be transmitted from humans to cattle in three cases in the USA.⁸⁹ In Poland, inter-species transmission of *M. bovis* between a farmer and his cattle has been reported.¹²⁶ Other WGS studies have been used to exhibit the dynamic transmission of *M. bovis* isolates between cattle and wildlife populations in different epidemiological settings, including in the UK,¹²⁷ Ireland,¹²⁸ New Zealand,⁷⁹ United States of America,¹²³ Mexico,¹²⁹ Italy,¹³⁰ Spain¹³¹ and Bulgaria.¹³² All the above demonstrate the value of WGS research of *M. bovis* transmission at the livestock–wildlife interface, presenting insights into bTB control.^{133,134} Fig. 3 shows the interspecies transmission of bTB for livestock–human–wildlife.

WGS insights into the genetic diversity of *M. bovis*

Knowledge of the genetic diversity of *M. bovis* is necessary for detecting its routes of transmission.¹³⁵ Single nucleotide polymorphism (SNP) analysis within virulence genes, which



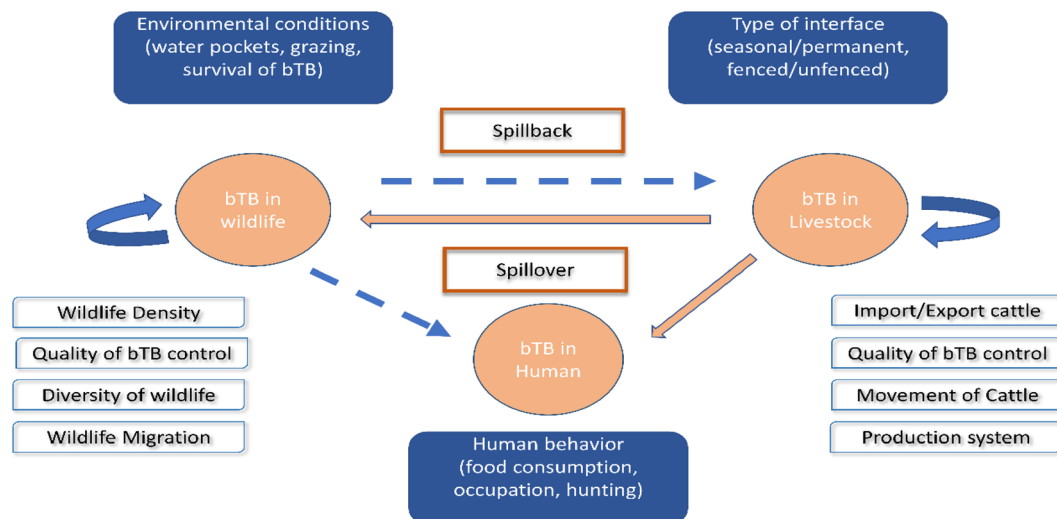


Fig. 3 Interspecies transmission of bTB for livestock–human–wildlife. The bTB can remain in livestock and in wildlife and is encouraged by the different factors included in the white boxes. The danger of bTB is spillover from livestock to human or wildlife, as well as spillback from wildlife to livestock, as shown in the blue boxes.¹¹⁹

appear with the highest number of SNPs, indicated that *M. bovis* was continuously modifying and adapting specific mechanisms to sustain dissemination among environments and different hosts.¹³⁶ The identification of non-synonymous SNPs on the virulence genes allowed for the differentiation of various groups.¹³⁷ The Mycomar T7 phagemid delivery system was used to produce whole genome transposon libraries in laboratory strains of *M. bovis* and *M. tuberculosis* species to compare the gene status during exposure to identical *in vitro* conditions. In addition, CRISPRi was successfully utilized in these two species to determine the effects of silencing genes with Rv2182c/Mb2204c, a gene included in glycerophospholipid metabolism and wag31, a gene involved in peptidoglycan synthesis, and the results showed that inhibition expression of Mb2204c in *M. bovis* displayed a considerably smaller growth effect than silencing its orthologue (Rv2182c) in *M. tuberculosis*. Given that glycerophospholipid metabolism is a confirmed pathway for antimicrobials.¹³⁷ Furthermore, a comparative genomic analysis of MTBC strains focused on the three features of pathogenicity: virulence, epitope variations, and host association, and the results provided insights into the pathogenic mechanisms, which helps with prevention, management and treatment of the disease.¹³⁸

The pan-genome is made up of all of a species' genes, and its examination is a very instructive method that allows stratification into: (1) the core genome containing genes that all strains share; (2) the accessory genome containing unnecessary genes not found in all strains; (3) singletons, which are distinct genes that are peculiar to a given strain. The core-genome controls the fundamental phenotype and contains the genes required for the biological processes. The accessory genome is important for genetic diversity of species and adaptation to various environmental survival, new hosts, immune evasion or other tasks that are advantageous over other species.¹³⁹ A comparative analysis

of 70 *M. bovis* genomes revealed the core and accessory genome components; with 2736 CDS for the former, while the accessory part consists of 3897 CDS, of which 2656 are confined to one/two genomes only. The functional annotation of that genome analysis categorized each CDS into one or several COG (clusters of orthologous groups) categories; lipid metabolism ($n = 242$), transcription ($n = 258$), unknown function ($n = 876$) and energy production ($n = 214$). Another study disclosed *M. bovis* lineages by applying pangenome analysis, and the results showed 3900 core genes and only 96 accessory genes.⁴⁸

Ceres and other scientists have proven that: (1) the accessory genome of *M. bovis* is noticeably smaller than previously believed, and this finding supports continuing clonal evolution and a closed pangenome with low gene content fluctuation throughout outbreaks; (2) several genes have frameshift mutations, including glpK, which has recently been linked to antibiotic tolerance in *M. tuberculosis*; (3) indels are the primary cause of the minor gene content variation that develops over brief time periods among closely related sequences; (4) multiple genes have potentially reversible homopolymeric tract alterations, indicating that phase variation may be a common method of gene regulation in MTBC.¹⁴⁰

Nanotechnology based targeted drug delivery systems

Nanotechnology is considered a potential tool for the diagnosis and treatment of disease. Nanoparticles can be used as appropriate displacers of the traditional drugs used for the treatment of tuberculosis. Nanocapsules containing antimicrobial drugs are instantly taken up by macrophages, which play an important role, to efficiently reach the active targeted sites.¹⁴¹ Recently, *M. tuberculosis* DNA biomarker and biosensing platform studies have been involved in the rapid diagnosis of TB and rifampicin-resistant TB (RR-TB).^{142,143} Several anti-TB agent-



based nanomaterials have attracted much attention to be applied for rapid diagnosis and effective treatment, encompassing noble metals (e.g. Au, Ag, Zn), semi-conducting materials such as metal oxides, and carbon nanotubes.¹⁴⁴ These nanomaterials showed a high tendency to reveal the signal after recognizing the targeted DNA biomarker.¹⁴⁴

Fig. 4 shows a summary of different nanotechnology based targeted drug delivery systems.

Nanoparticles for electrochemical detection of *H. tuberculosis*

Various biochemical methods including physical examination as well as sputum smear microscopy, chest photofluorography, and nucleic acid amplification tests have been used for the assessment of *H. tuberculosis*.¹⁴⁵ Unfortunately, these methods are considered complicated, expensive, and time consuming. Thus, there has been a great need for developing novel methods and techniques for efficient determination of *H. tuberculosis*. Recently, electrochemical sensors have been investigated as a rapid tool for the diagnosis of tuberculosis. For example, gold particle-decorated graphene as modified paper has been used for the rapid diagnosis of tuberculosis.¹⁴⁶ Additionally, rapid electrochemical detection of tuberculosis has been achieved by measuring Ag85 activity with disposable carbon sensors.¹⁴⁷

NP-based electrochemical sensors have attracted attention, especially in the biomedical field, because of their high sensitivity, stability, and selectivity.¹⁴⁸ Various electrochemical sensors including voltammetric, amperometric, and impedimetric sensors have been extensively investigated for targeting

many organic and inorganic pollutants as well as biological molecules and drugs.²³ In the present review, we highlight the role of different NP-based electrochemical sensors as valuable tools for targeting *H. tuberculosis*. The most reported electrochemical sensors used for *H. tuberculosis* are based on bio-sensing strategies. These strategies depend on using biological components as recognition elements. The NPs are used for enhancing the performance of these biosensors. They can be used as reporter probes or as enhancers of the performance of other redox probes. To the best of our knowledge, only one study has reported the use of NPs as recognition elements for *H. tuberculosis*.¹⁴⁵

A DNA-based sensor or aptasensor depends on the formation of a DNA double helix between the probe and the target DNA. The hybridization results in signal amplification or inhibition of the NPs. This signal can be monitored using voltammetric techniques such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV), or the electrochemical impedance spectroscopy (EIS) technique. The amplification signal of voltammetric techniques and the inhibition signal of EIS are attributed to the enhancement of the conductivity or decrease of the impedance at the electrode surface, respectively. In contrast, the inhibition signal of voltammetric techniques and amplification signal of EIS are attributed to the decrease of the conductivity or increase of the impedance at the electrode surface, respectively (Fig. 5). Similarly, antigen-antibody-based sensors or immunosensors are based on the interaction of antigens immobilized on the electrode surface and the antibody of *H. tuberculosis*. This interaction affects the oxidation current of the NP redox probe at the electrode surface. Different NP-based electrochemical sensors used for targeting *H. tuberculosis* are listed in Table 2.

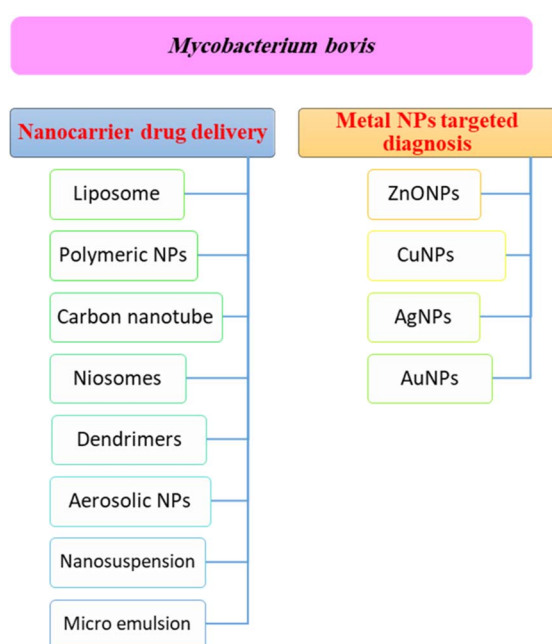


Fig. 4 A summary of different nanotechnology based targeted drug delivery systems. The blue color represents the different nanocarrier drug delivery methods while the orange color represents the metal nanoparticle targeted diagnosis of *M. bovis*.

Gold nanoparticles (AuNPs) for electrochemical detection of *H. tuberculosis*

AuNPs are considered to be excellent electrode modifiers because of their stability and superior conductivity. Additionally, they can improve the sensitivity of electrochemical sensors.^{149–151} Various AuNP-based electrochemical sensors have been used for detecting *H. tuberculosis* (Table 2). For example, dextrin-AuNPs were previously studied as an electrochemical reporter for detecting *M. tuberculosis* using a DNA-based biosensor.¹⁵² The sensing strategy is based on the electrochemical detection of AuNPs. Additionally, *N*-(aminobutyl)-*N*-(ethylisoluminol) functionalized AuNPs as an electrochemical label were used for the detection of *M. tuberculosis* using electrogenerated chemiluminescence (EIS).¹⁵³ Recently, an electrochemical sensor fabricated using the layer-by-layer assembly of AuNPs, polyethylenimine (PEI), and chondroitin sulfate (CS) has been used for the selective determination of the genes of *M. tuberculosis* using the DPV technique.¹⁵⁴ The synergistic effect of PEI and CS enhance the antifouling properties of the sensor towards single proteins. The sensor performance is based on the signal amplification strategy. Additionally, AuNPs modified with 5'SH-ssDNA have been immobilized over reduced graphene oxide nanoribbons for the electrochemical detection of



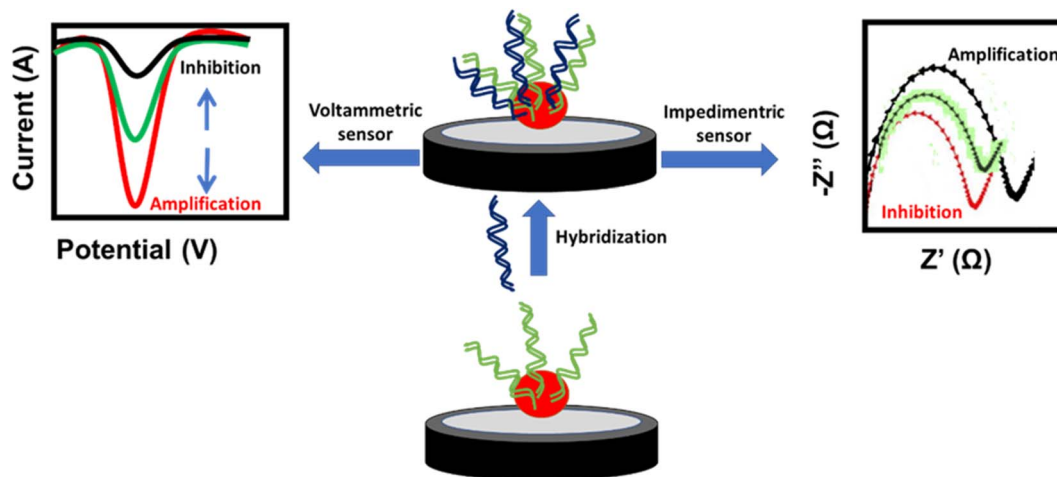


Fig. 5 Schematic representation of the sensing mechanisms of DNA-based sensors.

M. tuberculosis using the CV technique.¹⁵⁵ On the other hand, urchin-like Ce-MOFs have been used for loading large amounts of AuNPs on the surface of GCE leading to dense immobilization of the capture probe. The mechanism of sensing was based on signal amplification using anthraquinone-2-carboxylic acid as a redox nanoprobe.¹⁵⁶

Silver nanoparticles (AgNPs) for the electrochemical detection of *H. tuberculosis*

Silver nanoparticles (AgNPs) have been extensively investigated as recognition and redox probes for detecting inorganic and organic molecules.^{23,157–159} Interestingly, they have been studied as electrode modifiers for detecting *M. tuberculosis*. For example, GCE modified with a nanotriplex of graphene quantum dots, Fe₃O₄, and AgNPs has been reported as a novel electrochemical immunosensor of *M. tuberculosis*, where AgNPs prevent the aggregation of Fe₃O₄ and enhance the electrical conductivity, which facilitates the electron transport at the electrode surface.¹⁶⁰

Copper nanoparticles (CuNPs) for the electrochemical detection of *H. tuberculosis*

Copper nanoparticles (CuNPs) are a promising candidate for fabricating electrochemical sensors due to their high surface area and strong adsorption capability, as well as redox and catalytic properties.^{161–163} Interestingly, CuNPs have been used for enhancing the electron transfer at the electrode surface for detecting *H. tuberculosis* DNA. First, CuNPs were electrochemically deposited at the platinum electrode using the chronoamperometric technique, where Cu²⁺ was reduced at -0.39 V. After electrode modification, ssDNA was immobilized on the CuNPs *via* incubation, then 6-mercapto-1-hexanol was utilized for the construction of a well-aligned DNA monolayer. The sensing principal is based on the change of the ferrocyanide oxidation current after the hybridization of the ssDNA immobilized on the modified electrode with its complementary DNA.

The signal inhibition of the ferrocyanide redox probe upon increasing the concentration of the DNA was used for plotting the calibration curve.¹⁶⁴

Cobalt nanoparticles (CoNPs) for the electrochemical detection of *H. tuberculosis*

Cobalt nanoparticles (CoNPs), especially those fabricated using biological molecules, have exceptional stability, good electroactive sites and electrical conductivity, which make them an appropriate candidate for many electrochemical sensing applications.^{165,166} They have been used as recognition probes for detecting methyl nicotinate, which is a metabolite of *M. tuberculosis*. The CoNPs were incorporated with reduced graphene oxide (rGO) dispersed N-doped phenolic polymer precursor-based carbon film for constructing the sensor. Additionally, the carbon film was doped with N-heteroatom using melamine to increase the conductivity of the film.¹⁴⁵

Magnetic NPs for the electrochemical detection of *H. tuberculosis*

Magnetic nanoparticles have useful features such as large surface area, low toxicity, small particle size, and superparamagnetic behavior, which make them an excellent electrode modifier for constructing electrochemical sensors.^{167,168} An electrochemical sensor based on Fe₃O₄ incorporated with polypyrrole and a naphthoquinone probe was previously constructed for detecting *H. tuberculosis*. For modifying the surface of the gold screen-printed electrode with the magnetic particles, a permanent magnet was used, and the potential was swept from -0.4 to $+0.8$ V *vs.* Ag/AgCl reference electrode with a scan rate of 50 mV s⁻¹ for ten cycles. The presence of magnetic nanoparticles enhances the response of the redox probe. The hybridization between a probe and the complementary DNA target led to a large decrease in the naphthoquinone redox oxidation current which was monitored using the SWV technique.¹⁶⁹



Table 2 NP-based electrochemical sensors for detecting *H. tuberculosis*^a

NP	Modified electrode	NP-modification technique	Type of sensor	Sensing technique	Sensing mechanism	LOD	Ref.
Au	ABE/AuNPs/Au electrode	Drop casting	Aptasensor	ECL	Signal amplification	3.3×10^{-16} M	153
	AuNPs/Ce-MOFs/GCE	Electrochemical reduction	Aptasensor	DPV	Signal amplification	67.6 fg mL^{-1}	156
	Dextrin-AuNPs/SPCE	Drop casting	Aptasensor	DPV	Signal amplification	$0.01 \text{ ng } \mu\text{L}^{-1}$	152
	AuNPs/PEI/CS/GCE	Electrochemical reduction	Aptasensor	DPV	Signal inhibition	0.03 fM	154
Ag	rGO nanoribbons/AuNPs/ITO	Drop casting	Aptasensor	CV	Signal amplification	0.1 fM	155
	Graphene quantum dots/Fe ₃ O ₄ /AgNPs/GCE	Drop casting	Immunosensor	DPV	Signal amplification	0.33 ng mL^{-1}	160
	CuNPs/Pt electrode	Electrochemical reduction	Aptasensor	DPV	Signal inhibition	10^{-9} M	164
	CoNPs/rGO	Drop casting	Non-biological sensor	DPV	Signal inhibition	4×10^{-7} M	145
Fe ₃ O ₄	PPY-Fe ₃ O ₄ /AuSPE	Adsorption in presence of magnet	Aptasensor	SWV	Signal inhibition	1 fM	169
Graphene	GNP/SPGE	Electrolytic exfoliation method	Genosensor	CV	Signal inhibition	1 pg	170
PPY nanowire	PPY nanowire/Au electrode	Electrodeposition	Aptasensor	SWV	Signal inhibition	3.6×10^{-19} M	175
MWCNTs	HAPNPs/PPY/MWCNTs/GCE	Drop casting	Aptasensor	DPV	Signal amplification	0.141×10^{-9} M	177

^a ABEI: *N*-(aminobutyl)-*N*-(ethylisoluminol), AuNPs: gold nanoparticles, AuSPE: gold screen-printed electrode, ECL: electrogenerated chemiluminescence, CS: chondroitin sulfate, CV: cyclic voltammetry, DPV: differential pulse voltammetry, GCE: glassy carbon electrode, GNP: graphene nanoparticle powder, HAPNPs: hydroxyapatite nanoparticles, ITO: indium tin oxide, LOD: limit of detection, MWCNTs: multi-walled carbon nanotubes, rGO: reduced graphene oxide, PEI: polyethylenimine, PPY: polypyrrole, SPGE: screen-printed graphene electrode, SWV: square wave voltammetry.

Graphene NPs for the electrochemical detection of *H. tuberculosis*

Graphene is considered an attractive material for the biosensing process due to its superb structural, optical, and electronic features, as well as its biocompatibility and mechanical stability.¹⁷⁰ Graphene-based nanomaterials have been widely used in sensing applications, both directly or as substrates for other materials.¹⁷¹ Previously, a screen printed graphene electrode has been modified with graphene NPs using the electrolytic exfoliation method for targeting the DNA of *H. tuberculosis*. The presence of graphene NPs increases the electron transfer at the electrode surface.¹⁷⁰

Polypyrrole nanowires for the electrochemical detection of *H. tuberculosis*

Polypyrrole has been widely used due to its good environmental stability and extraction ability of polar compounds.¹⁷² Polypyrrole nanowires are applied in many applications due to their good chemical and physical properties.^{173,174} The features of polypyrrole nanowires, such as the hydrophilic character and the large surface area, enhance the electron transfer at the electrode surface. They have been previously used for enhancing the performance of the ferrocenyl group as a redox probe for detecting the genomic DNA of *H. tuberculosis*. The sensing principal is based on the decrease of the redox current of the ferrocenyl oxidation, which was monitored using the SWV technique. This decrease is attributed to the small amount of electron transfer occurring after the hybridization of the complementary DNA and the formation of a double strand on the surface.¹⁷⁵

Multi-walled carbon nanotubes (MWCNTs) for the electrochemical detection of *H. tuberculosis*

Carbon-based electrode materials with diverse porous structures show enhanced electrochemical sensing abilities due to their superior conductivity, as well as the absorption and enrichment of analytes from porous structures.¹⁷⁶ Previously, a PCR-free DNA electrochemical biosensor was constructed based on multi-walled carbon nanotubes (MWCNTs), polypyrrole, and hydroxyapatite NPs for detecting the DNA of *H. tuberculosis*. MWCNTs were essential for improving the electrical conductivity, increasing the surface area of the electrode, and providing an ideal immobilization material for the DNA of *H. tuberculosis*. The sensing principal was based on signal amplification, which was monitored using DPV.¹⁷⁷

Conclusion

bTB is an important zoonotic disease that threatens humans and animals worldwide. *M. bovis* is considered a spillover pathogen, which can be transmitted from livestock to humans to wildlife. More molecular epidemiological studies are needed to determine the dynamic transmission of bTB, to define the dominant genotypes between humans and animals, and to understand the phylogenetic relationships of the strains. Spoligotyping and MIRU-VNTR are widely used to discriminate

between *M. bovis* isolates. Some of the disadvantages of these two techniques are homoplasmy and lower resolution power in spoligotyping, and WGS solved these problems. WGS is a valuable tool due to its high-resolution for the analysis of *M. bovis* genomic diversity, and it provides insights into the role of recombination and positive selection as evolutionary driving forces in a pathogen affecting a large range of host species, with economical and biodiversity impacts across the world. The current review also summarizes the NP-based electrochemical sensors that have been used for detecting tuberculosis. Notably, most of the reported electrochemical sensors are based on biological elements as recognition probes. NPs act as reporter probes or as enhancers of the performance of other redox probes. The sensing principal is based on tuberculosis DNA (aptasensor), antigens (immunosensor), or biomarkers. The aptasensor depends on the formation of a DNA double helix between the probe and the target DNA leading to signal amplification or inhibition of the NPs which can be monitored by voltammetric and impedimetric methods. The immunosensor is based on the interaction of antigens immobilized on the electrode surface and the antibodies of tuberculosis. Overall, NP-electrochemical sensors present an efficient tool for the detection of tuberculosis. They offer a good opportunity for the simple, sensitive, and rapid detection of tuberculosis. However, there can be some challenges, such as the occurrence of non-specific binding with other biological molecules in real-life applications. Therefore, we recommend the integration of microfluidics with electrochemical sensors for reliable results.

Conflicts of interest

There are no conflicts to declare.

Notes and references

- G. Matope, M. B. Gadaga, B. Bhebhe, P. T. Tshabalala and P. V. Makaya, *Vet. Med. Sci.*, 2023, **9**, 1327–1337.
- A. P. Barnes, A. Moxey, S. Brocklehurst, A. Barratt, I. J. McKendrick, G. Innocent and B. V. Ahmadi, *Prev. Vet. Med.*, 2023, **211**, 105808.
- F. Admassu, E. Abera, A. Gizachew, T. Sedoru and T. Gari, *BMJ Open*, 2023, **13**, e061836.
- K. Murai, P. Tizzani, L. Awada, L. Mur, N. J. Mapitse and P. C  ceres, *Bulletin de l'OIE*, 2019, **2019**, 1–3.
- O. Andrievskaia, A. Garceac, D. Lloyd, M. Savic and M.-O. Duceppe, *Microbiol. Resour. Announce.*, 2023, **12**, e0121322.
- P. R. Sichewo, T. M. Hlokwe, E. M. C. Etter and A. L. Michel, *BMC Microbiol.*, 2020, **20**, 1–9.
- T. T. Deneke, A. Bekele, H. L. Moore, T. Mamo, G. Almaw, G. A. Mekonnen, A. Mihret, R. Tschopp, L. Yeheyis, C. Hodge, J. L. N. Wood, S. Berg, A. Aseffa, B. Tessema, B. Belachew, E. Fekadu, F. Melese, G. Gemechu, H. Taye, S. Haile, S. Ayalew, T. Hailu, C. Yirga, M. Ambaw, T. Solomon, T. Teklewold, S. Gebre, G. Gari, M. Sahle, A. Aliy, A. Olani, A. Sirak, G. Mekonnen, M. Tamiru, S. Guta, J. Wood, A. Conlan, A. Clarke, C. Smith, R. G. Hewinson, M. Vordermeier, J. Nunez-Garcia, G. Ameni, B. Bayissa, A. Zewude, A. Worku, L. Terfassa, M. Chanyalew, T. Mohammed and M. Zeleke, *BMC Public Health*, 2022, **22**, 222.
- M. Krajewska-W  dzina,  . Radulski, W. R. Waters, A. Didkowska, A. Zabost, E. Augustynowicz-Kope  , S. Brzezi  ska and M. Weiner, *Pathogens*, 2022, **11**, 1170.
- R. Gopalaswamy, V. N. A. Dusthacker, S. Kannayan and S. Subbian, *Journal of Respiration*, 2021, **1**, 141–164.
- B. M  ller, S. D  rr, S. Alonso, J. Hattendorf, C. J. M. Laisse, S. D. C. Parsons, P. D. van Helden and J. Zinsstag, *Emerging Infect. Dis.*, 2013, **19**, 899–908.
- E. Borja, L. F. Borja, R. Prasad, T. Tunabuna and J. A. L. M. L. Toribio, *Front. Vet. Sci.*, 2018, **5**, 270.
- S. Niemann and P. Supply, *Cold Spring Harbor Perspect. Med.*, 2014, **4**, a021188.
- A. C. Sch  rch and D. van Soolingen, *Infect., Genet. Evol.*, 2012, **12**, 602–609.
- A. Roetzer, R. Diel, T. A. Kohl, C. R  ckert, U. N  bel, J. Blom, T. Wirth, S. Jaenicke, S. Schuback and S. R  sch-Gerdes, *PLoS Med.*, 2013, **10**, e1001387.
- D. Couvin, I. Cervera-Marzal, A. David, Y. Reynaud and N. Rastogi, *Database*, 2022, **2022**, 1–8.
- F. Tazerart, J. Saad, N. Sahraoui, D. Yala, A. Niar and M. Drancourt, *Pathogens*, 2021, **10**, 802.
- R. Avoi and Y. C. Liaw, *Int. J. Environ. Res. Public Health*, 2021, **18**, 9740.
- A. El-Sayed, S. El-Shannat, M. Kamel, M. A. Casta  eda-Vazquez and H. Casta  eda-Vazquez, *Zoonoses Public Health*, 2016, **63**, 251–264.
-  . R  veillaud, S. Desvaux, M. L. Boschirolu, J. Hars,  . Faure, A. Fediaevsky, L. Cavalerie, F. Chevalier, P. Jabert, S. Poliak, I. Tourette, P. Hendrikx and C. Richomme, *Front. Vet. Sci.*, 2018, **5**, 262.
- M. Zahran, *Heliyon*, 2023, e19943.
- Z. Khalifa, M. Zahran, M. A. Zahran and M. A. Azzem, *RSC Adv.*, 2020, **10**, 37675–37682.
- O. Simoska and K. J. Stevenson, *Analyst*, 2019, **144**, 6461–6478.
- M. M. Zahran, Z. Khalifa, M. Zahran and M. A. Azzem, *Mater. Adv.*, 2021, 7350–7365.
- H. Zhang, M. Liu, W. Fan, S. Sun and X. Fan, *Front. Public Health*, 2022, **10**, 994745.
- M. Lasserre, P. Fresia, G. Greif, G. Iraola, M. Castro-Ramos, A. Juambeltz,  . Nu  ez, H. Naya, C. Robello and L. Bern  , *BMC Genomics*, 2018, **19**, 2.
- D. Brites, C. Loiseau, F. Menardo, S. Borrell, M. B. Boniotti, R. Warren, A. Dippenaar, S. D. C. Parsons, C. Beisel, M. A. Behr, J. A. Fyfe, M. Coscolla and S. Gagneux, *Front. Microbiol.*, 2018, **9**, 2820.
- S. Gagneux, *Nat. Rev. Microbiol.*, 2018, **16**, 202–213.
- C. V. Mulholland, A. C. Shockey, H. L. Aung, R. T. Cursons, R. F. O'Toole, S. S. Gautam, D. Brites, S. Gagneux, S. A. Roberts, N. Karalus, G. M. Cook, C. S. Pepperell and V. L. Arcus, *Front. Microbiol.*, 2019, **10**, 2778.
- Z. Liu, Z. Jiang, W. Wu, X. Xu, Y. Ma, X. Guo, S. Zhang and Q. Sun, *Front. Microbiol.*, 2022, **13**, 3419.



- 30 H. Ru, X. Liu, C. Lin, J. Yang, F. Chen, R. Sun, L. Zhang and J. Liu, *Front. Cell. Infect. Microbiol.*, 2017, **7**, 239.
- 31 D. Bespiatykh, J. Bespiatykh, I. Mokrousov and E. Shitikov, *mSphere*, 2021, **6**, e0053521.
- 32 R. M. Warren, N. C. Gey van Pittius, M. Barnard, A. Hesselning, E. Engelke, M. de Kock, M. C. Gutierrez, G. K. Chege, T. C. Victor, E. G. Hoal and P. D. van Helden, *Int. J. Tuberc. Lung Dis.*, 2006, **10**, 818–822.
- 33 C. K. Zimpel, P. E. Brandão, A. F. de Souza Filho, R. F. de Souza, C. Y. Ikuta, J. S. F. Neto, N. C. Soler Camargo, M. B. Heinemann and A. M. S. Guimarães, *Front. Microbiol.*, 2017, **8**, 2389.
- 34 G. Delogu, M. J. Brennan and R. Manganelli, *Adv. Exp. Med. Biol.*, 2017, **1019**, 191–207.
- 35 C. K. Zimpel, J. S. L. Patané, A. C. P. Guedes, R. F. de Souza, T. T. Silva-Pereira, N. C. S. Camargo, A. F. de Souza Filho, C. Y. Ikuta, J. S. F. Neto, J. C. Setubal, M. B. Heinemann and A. M. S. Guimaraes, *Front. Microbiol.*, 2020, **11**, 843.
- 36 O. K. Tørresen, B. Star, P. Mier, M. A. Andrade-Navarro, A. Bateman, P. Jarnot, A. Gruca, M. Grynberg, A. V. Kajava, V. J. Promponas, M. Anisimova, K. S. Jakobsen and D. Linke, *Nucleic Acids Res.*, 2019, **47**, 10994–11006.
- 37 D. M. Collins and G. W. De Lisle, *J. Gen. Microbiol.*, 1984, **130**, 1019–1021.
- 38 R. Hodgeman, R. Mann, K. Savin, N. Djitro, S. Rochfort and B. Rodoni, *BMC Microbiol.*, 2021, **21**, 1–16.
- 39 M. Price-Carter, S. Rooker and D. M. Collins, *Vet. Microbiol.*, 2011, **150**, 107–114.
- 40 H. m. Neoh, X. E. Tan, H. F. Sapri and T. L. Tan, *Infect., Genet. Evol.*, 2019, **74**, 103935.
- 41 C. Ghazaei, *J. Res. Med. Sci.*, 2018, **23**, 63.
- 42 S. Jeon, N. Lim, S. Park, M. Park and S. Kim, *J. Microbiol. Biotechnol.*, 2018, **28**, 338–346.
- 43 H. Ravansalar, K. Tadayon and K. Ghazvini, *Iran. J. Microbiol.*, 2016, **8**, 338.
- 44 A. Kechin, I. Ostorbin, A. Cherednichenko, E. Khrapov, Y. Schwartz, N. Stavitskaya and M. Filipenko, *Arch. Microbiol.*, 2023, **205**, 71.
- 45 H. Alonso, J. I. Aguilo, S. Samper, J. A. Caminero, M. I. Campos-Herrero, B. Gicquel, R. Brosch, C. Martín and I. Otal, *Tuberculosis*, 2011, **91**, 117–126.
- 46 T. Roychowdhury, S. Mandal and A. Bhattacharya, *Sci. Rep.*, 2015, **5**, 1–10.
- 47 C. Y. Soto, M. C. Menéndez, E. Pérez, S. Samper, A. B. Gómez, M. J. García and C. Martín, *J. Clin. Microbiol.*, 2004, **42**, 212–219.
- 48 C. Charles, C. Conde, F. Biet, M. L. Boschirololi and L. Michelet, *Front. Microbiol.*, 2022, **13**, 1868.
- 49 H. Alonso, S. Samper, C. Martín and I. Otal, *BMC Genomics*, 2013, **14**, 422.
- 50 K. Kremer, J. R. Glynn, T. Lillebaek, S. Niemann, N. E. Kurepina, B. N. Kreiswirth, P. J. Bifani and D. Van Soolingen, *J. Clin. Microbiol.*, 2004, **42**, 4040–4049.
- 51 C. J. Augusto, W. d. S. Carvalho, I. N. d. Almeida, L. J. d. A. Figueiredo, N. G. T. Dantas, P. N. Suffys and S. S. d. Miranda, *Braz. J. Microbiol.*, 2018, **49**, 641–646.
- 52 S. M. d. Almeida, A. C. Malaspina, C. Q. F. Leite and M. H. F. Saad, *Rev. do Inst. Med. Trop. São Paulo*, 2019, **61**, e10.
- 53 E. Green, L. C. Obi, A. I. Okoh, M. Nchabeleng, B. E. De Villiers, T. Letsoalo, A. A. Hoosen, P. O. Bessong and R. N. Ndip, *J. Health Popul. Nutr.*, 2013, **31**, 1.
- 54 J. Gonzalo-Asensio, I. Pérez, N. Aguiló, S. Uranga, A. Picó, C. Lampreave, A. Cebollada, I. Otal, S. Samper and C. Martín, *PLoS Genet.*, 2018, **14**, e1007282.
- 55 S. Thabet, A. Karboul, N. Dekhil and H. Mardassi, *Int. J. Infect. Dis.*, 2014, **29**, 211–218.
- 56 B. G. Eimani, M. Seyyedi, L. Sahebi, A. Monfaredan and M. Khalili, *Int. J. Mycobact.*, 2015, **4**, 123.
- 57 S. Desikan and S. Narayanan, *Indian J. Med. Res.*, 2015, **141**, 761.
- 58 X. Zeng, H. Li, R. Zheng, N. Kurepina, B. N. Kreiswirth, X. Zhao, Y. Xu and Q. Lia, *J. Clin. Microbiol.*, 2016, **54**, 2384.
- 59 C. Genestet, E. Hodille, A. Bernard, M. Vallée, G. Lina, A. Le Meur, G. Refrégier and O. Dumitrescu, *Microbiol. Spectrum*, 2022, **10**, e0022322.
- 60 J. Zhang, E. Abadia, G. Refregier, S. Tafaj, M. L. Boschirololi, B. Guillard, A. Andremon, R. Ruimy and C. Sola, *J. Med. Microbiol.*, 2010, **59**, 285–294.
- 61 N. H. Smith, S. V. Gordon, R. de la Rua-Domenech, R. S. Clifton-Hadley and R. G. Hewinson, *Nat. Rev. Microbiol.*, 2006, **4**, 670–681.
- 62 A. G. M. Van Der Zanden, A. H. Hoentjen, F. G. C. Heilmann, E. F. Weltevreden, L. M. Schouls and J. D. A. Van Embden, *Mol. Pathol.*, 1998, **51**, 209.
- 63 K. Masoud, G. F. Araj, L. Reslan, S. Fadlallah, M. Wehbe, L. Y. Itani, A. Z. Avedissian, G. Dbaiibo, A. Saade, G. Refrégier, C. Sola and G. M. Matar, *J. Infect. Dev. Countries*, 2020, **14**, 878–885.
- 64 V. Lorente-Leal, E. Liandris, J. Bezos, M. Pérez-Sancho, B. Romero and L. d. Juan, *Front. Vet. Sci.*, 2022, **9**, 827702.
- 65 A. M. Cabibbe, P. Miotto, R. Moure, F. Alcaide, S. Feuerriegel, G. Pozzi, V. Nikolayevskyy, F. Drobniewski, S. Niemann, K. Reither, D. M. Cirillo, P. Di Pietro, F. San Biagio, E. Alessi, T. G. Barbuzzi, S. Tafaj, E. Bachyska, I. Kontsevaya, Y. Balabanova, E. Lazzeri, J. Sserunkuma, F. Aloï, M. Nsubuga and M. Sasamalo, *J. Clin. Microbiol.*, 2015, **53**, 3876–3880.
- 66 J. Li, Y. Wang, L. Yan, C. Zhang, Y. He, J. Zou, Y. Zhou, C. Zhong and X. Zhang, *Microbes Infect.*, 2022, **24**, 105002.
- 67 S. Kulkarni, C. Sola, I. Filliol, N. Rastogi and G. Kadival, *Res. Microbiol.*, 2005, **156**, 588–596.
- 68 J. R. Driscoll, M. A. McGarry and H. W. Taber, *J. Clin. Microbiol.*, 1999, **37**, 274.
- 69 J. F. Reyes, C. H. S. Chan and M. M. Tanaka, *Infect. Genet. Evol.*, 2012, **12**, 811–818.
- 70 A. M. S. Guimaraes and C. K. Zimpel, *Microorganisms*, 2020, **8**, 667.
- 71 R. Ramazanzadeh and R. McNerney, *Pak. J. Biol. Sci.*, 2007, **10**, 2612–2621.
- 72 M. Ghavidel, K. Tadayon, N. Mosavari, K. Nourian, H. R. BahramiTaghanaki, G. R. Mohammadi,



- M. Rashtibaf and K. Ghazvini, *Rep. Biochem. Mol. Biol.*, 2019, **8**, 335.
- 73 M. Kargarpour Kamakoli, S. Hadifar, S. Khanipour, G. Farmanfarmaei, A. Fateh, S. D. Siadat and F. Vaziri, *Infect. Dis.*, 2019, **51**, 659–667.
- 74 M. Kato-Maeda, J. Z. Metcalfe and L. Flores, *Future Microbiol.*, 2011, **6**, 203–216.
- 75 T. Jagielski, J. Van Ingen, N. Rastogi, J. Dziadek, P. K. Mazur and J. Bielecki, *BioMed Res. Int.*, 2014, **2014**, 645802.
- 76 D. H. Wyllie, J. A. Davidson, E. Grace Smith, P. Rathod, D. W. Crook, T. E. A. Peto, E. Robinson, T. Walker and C. Campbell, *EBioMedicine*, 2018, **34**, 122–130.
- 77 F. Belakehal, S. A. Barth, C. Menge, H. T. Mossadak, N. Malek and I. Moser, *PLoS One*, 2022, **17**, e0262390.
- 78 É. B. Sales, A. A. Fonseca, C. M. Gonçalves, A. P. Lage, G. I. Andrade, P. N. Suffys, H. M. Gomes, N. L. Dias, J. S. Ferreira Neto, A. M. d. S. Guimarães and M. B. Heinemann, *Front. Vet. Sci.*, 2021, **8**, 396.
- 79 M. Price-Carter, R. Brauning, G. W. de Lisle, P. Livingstone, M. Neill, J. Sinclair, B. Paterson, G. Atkinson, G. Knowles, K. Crews, J. Crispell, R. Kao, S. Robbe-Austerman, T. Stuber, J. Parkhill, J. Wood, S. Harris and D. M. Collins, *Front. Vet. Sci.*, 2018, **5**, 272.
- 80 Z. Djelouadji, C. Arnold, S. Gharbia, D. Raoult and M. Drancourt, *PLoS One*, 2008, **3**, e2433.
- 81 D. Kasir, N. Osman, A. Awik, I. El Ratel, R. Rafei, I. Al Kassaa, D. El Safadi, R. Salma, K. El Omari and K. J. Cummings, *Diseases*, 2023, **11**, 39.
- 82 P. R. Bapat, R. S. Dodkey, S. D. Shekhawat, A. A. Husain, A. R. Nayak, A. P. Kawle, H. F. Dagainawala, L. K. Singh and R. S. Kashyap, *Journal of Epidemiology and Global Health*, 2017, **7**, 277–283.
- 83 M. K. Ghebremariam, V. P. M. G. Rutten, J. C. M. Vernooij, K. Uqbazghi, T. Tesfaalem, T. Butsuamlak, A. M. Idris, M. Nielen and A. L. Michel, *BMC Vet. Res.*, 2016, **12**, 1–7.
- 84 H. Damene, D. Tahir, M. Diels, A. Berber, N. Sahraoui and L. Rigouts, *PLoS Neglected Trop. Dis.*, 2020, **14**, 1–18.
- 85 K. Tadayon, N. Mosavari, A. H. Shahmoradi, F. Sadeghi, A. Azarvandi and K. Forbes, *J. Vet. Med., Ser. B*, 2006, **53**, 41–42.
- 86 L. A. Dagher, J. Hassan, S. Kharroubi, H. Jaafar and I. I. Kassem, *Antibiotics*, 2021, **10**, 883.
- 87 I. I. Kassem and H. Jaafar, *Water Int.*, 2020, **45**, 423–429.
- 88 M. S. A. E. Elsayed, *Infect., Genet. Evol.*, 2019, **71**, 211–214.
- 89 J. E. Lombard, E. A. Patton, S. N. Gibbons-Burgener, R. F. Klos, J. L. Tans-Kersten, B. W. Carlson, S. J. Keller, D. J. Pritschet, S. Rollo, T. V. Dutcher, C. A. Young, W. C. Hench, T. C. Thacker, C. Perea, A. D. Lehmkuhl and S. Robbe-Austerman, *Front. Vet. Sci.*, 2021, **8**, 748.
- 90 A. Matos, L. Figueira, M. Martins, M. Matos, M. Morais, A. Dias and A. Coelho, *Vet. Rec.*, 2013, **173**, 376.
- 91 Y. F. Elnaker, M. S. Diab, N. A. Ibrahim, A. El-Gedawy, R. S. Zaki and A. Radwan, *Vet. World*, 2019, **12**, 1180.
- 92 G. S. Abdellazeq, M. M. Elnaggar, H. S. Osman, W. C. Davis and M. Singh, *Transboundary Emerging Dis.*, 2016, **63**, 497–507.
- 93 I. Elsohaby, H. A. Ahmed, M. M. El-Diasty, A. A. Elgedawy, E. Mahrous and F. I. El Hofy, *J. Appl. Microbiol.*, 2020, **129**, 1207–1219.
- 94 Y. F. Elnaker, N. T. Elsharawy, M. S. Daib, A. A. Elgedawy and N. A. Ibrahim, *Assiut Vet. Med. J.*, 2018, **64**, 179–186.
- 95 N. A. Hassanain, M. A. Hassanain, Y. A. Soliman, A. A. Ghazy and Y. A. Ghazy, *Adv. J. Microbiol. Res.*, 2014, 446–450, <https://internationalscholarsjournals.com>.
- 96 M. Lobna and E. Nasr, *Int. J. Adv. Res.*, 2015, **3**, 693–701.
- 97 M. Sabry and A. Elkerdasy, *J. Pharm. BioAllied Sci.*, 2014, **6**, 115–121.
- 98 S. Abbadi, G. El Hadidy, N. Gomaa and R. Cooksey, *Int. J. Infect. Dis.*, 2009, **13**, 236–242.
- 99 R. C. Cooksey, S. H. Abbadi, C. L. Woodley, D. Sikes, M. Wasfy, J. T. Crawford and F. Mahoney, *J. Clin. Microbiol.*, 2002, **40**, 1651–1655.
- 100 H. A. Abdelsadek, H. M. Sobhy, K. F. Mohamed, S. H. A. Hekal, A. N. Dapgh and A. S. Hakim, *Vet. World*, 2020, **13**, 2150.
- 101 Y. Yoshinaga, C. Daum, G. He and R. O'Malley, *Fungal Genomics: Methods and Protocols*, 2018, 37–52.
- 102 T. Zhang, J. Zhou, W. Gao, Y. Jia, Y. Wei and G. Wang, *Briefings Bioinf.*, 2022, **23**, bbac305.
- 103 R. Vaser, I. Sović, N. Nagarajan and M. Šikić, *Genome Res.*, 2017, **27**, 737–746.
- 104 M. Q. Wilber, K. M. Pepin, H. Campa, S. E. Hygnstrom, M. J. Lavelle, T. Xifara, K. C. VerCauteren and C. T. Webb, *J. Appl. Ecol.*, 2019, **56**, 1471–1481.
- 105 M. Domingo, E. Vidal and A. Marco, *Res. Vet. Sci.*, 2014, **97**, S20–S29.
- 106 B. Z. Katale, E. V. Mbugi, K. K. Siame, J. D. Keyyu, S. Kendall, R. R. Kazwala, H. M. Dockrell, R. D. Fyumagwa, A. L. Michel, M. Rweyemamu, E. M. Streicher, R. M. Warren, P. van Helden and M. I. Matee, *Transboundary Emerging Dis.*, 2017, **64**, 815.
- 107 M. V. Palmer, *Transboundary Emerging Dis.*, 2013, **60**, 1–13.
- 108 S. Berg, M. C. Garcia-Pelayo, B. Müller, E. Hailu, B. Asiimwe, K. Kremer, J. Dale, M. B. Boniotti, S. Rodriguez, M. Hilty, L. Rigouts, R. Firdessa, A. MacHado, C. Mucavele, B. N. R. Ngandolo, J. Bruchfeld, L. Boschioli, A. Müller, N. Sahraoui, M. Pacciarini, S. Cadmus, M. Joloba, D. Van Soolingen, A. L. Michel, B. Djonne, A. Aranaz, J. Zinsstag, P. Van Helden, F. Portaels, R. Kazwala, G. Källénus, R. G. Hewinson, A. Aseffa, S. V. Gordon and N. H. Smith, *J. Bacteriol.*, 2011, **193**, 670–678.
- 109 C. Gortázar, R. J. Delahay, R. A. McDonald, M. Boadella, G. J. Wilson, D. Gaviera-Widen and P. Acevedo, *Mamm. Rev.*, 2012, **42**, 193–206.
- 110 C. D. Foo, P. Shrestha, L. Wang, Q. Du, A. L. García-Basteiro, A. S. Abdullah and H. Legido-Quigley, *PLoS Med.*, 2022, **19**, e1003899.
- 111 I. D. Otchere, A. J. van Tonder, A. Asante-Poku, L. Sánchez-Busó, M. Coscollá, S. Osei-Wusu, P. Asare, S. Y. Aboagye, S. A. Ekuban, A. I. Yahayah, A. Forson, A. Baddoo, C. Laryea, J. Parkhill, S. R. Harris, S. Gagneux and D. Yeboah-Manu, *PLoS One*, 2019, **14**, e0209395.



- 112 H. F. M. Abdelaal, D. Spalink, A. Amer, H. Steinberg, E. A. Hashish, E. A. Nasr and A. M. Talaat, *Sci. Rep.*, 2019, **9**, 1–15.
- 113 T. G. Gökmen, G. Yakici, Y. Kalayci, N. Turut, M. M. Ocal, M. Haligür, E. Günaydin and F. Köksal, *Iran. J. Vet. Res.*, 2022, **23**, 210.
- 114 C. D'Souza, U. Kishore and A. G. Tsolaki, *Immunobiology*, 2022, **228**, 1–19.
- 115 O. F. Inlamea, P. Soares, C. Y. Ikuta, M. B. Heinemann, S. J. Achá, A. Machado, J. S. F. Neto, M. Correia-Neves and T. Rito, *PLoS Neglected Trop. Dis.*, 2020, **14**, e0008081.
- 116 M. Ejo, B. Haile, T. Tariku, S. Nigatu, E. Kebede, A. B. Bitew, Y. Demessie, G. Getaneh, A. Alebie, M. Girma, F. Ota and A. Nuru, *BMC Microbiol.*, 2021, **21**, 1–10.
- 117 S. A. Luciano and A. Roess, *Zoonoses Public Health*, 2020, **67**, 97.
- 118 H. Taye, K. Alemu, A. Mihret, J. L. N. Wood, Z. Shkedy, S. Berg and A. Aseffa, *Zoonoses Public Health*, 2021, **68**, 704–718.
- 119 A. V. Kwaghe, J. A. Ameh, C. A. Kudi, A. G. Ambali, H. K. Adesokan, V. O. Akinseye, O. D. Adelakun, J. G. Usman and S. I. Cadmus, *BMC Microbiol.*, 2023, **23**, 7.
- 120 A. V. Kwaghe, J. A. Ameh, C. A. Kudi, A. G. Ambali, H. K. Adesokan, V. O. Akinseye, O. D. Adelakun and J. G. Usman, *Molecular Characterization of Mycobacterium tuberculosis Complex in Cattle and Humans, Maiduguri, Borno State, Nigeria: a Cross-sectional Study*, 2022, DOI: [10.21203/RS.3.RS-1549668/V1](https://doi.org/10.21203/RS.3.RS-1549668/V1).
- 121 S. E. K. Acquah, P. Asare, E. K. Danso, P. Tetteh, A. Y. Tetteh, D. Boateng, S. Osei-Wusu, T. Afum, Y. I. Ayamdooh and E. A. Akugre, *PLoS Neglected Trop. Dis.*, 2022, **16**, e0010649.
- 122 S. E. Sandoval-Azuara, R. Muñiz-Salazar, R. Perea-Jacobo, S. Robbe-Austerman, A. Perera-Ortiz, G. López-Valencia, D. M. Bravo, A. Sanchez-Flores, D. Miranda-Guzmán, C. A. Flores-López, R. Zenteno-Cuevas, R. Laniado-Laborín, F. L. de la Cruz and T. P. Stuber, *Int. J. Infect. Dis.*, 2017, **63**, 48–56.
- 123 L. C. M. Salvador, D. J. O'Brien, M. K. Cosgrove, T. P. Stuber, A. M. Schooley, J. Crispell, S. V. Church, Y. T. Gröhn, S. Robbe-Austerman and R. R. Kao, *Mol. Ecol.*, 2019, **28**, 2192–2205.
- 124 D. M. Cooke, W. J. Goosen, T. Burgess, C. Witte and M. A. Miller, *Vet. Immunol. Immunopathol.*, 2023, **257**, 110559.
- 125 A. J. Van Tonder, M. J. Thornton, A. J. K. Conlan, K. A. Jolley, L. Goolding, A. P. Mitchell, J. Dale, E. Palkopoulou, P. J. Hogarth, R. G. Hewinson, J. L. N. Wood and J. Parkhill, *PLoS Pathog.*, 2021, **17**, e1010075.
- 126 M. Krajewska-Wędzina, Ł. Radulski, W. R. Waters, A. Didkowska, A. Zabost, E. Augustynowicz-Kopeć, S. Brzezińska and M. Weiner, *Pathogens*, 2022, **11**, 1170.
- 127 J. Crispell, C. H. Benton, D. Balaz, N. De Maio, A. Ahkmetova, A. Allen, R. Biek, E. L. Prescho, J. Dale, G. Hewinson, S. J. Lycett, J. Nunez-Garcia, R. A. Skuce, H. Trewby, D. J. Wilson, R. N. Zadoks, R. J. Delahay and R. R. Kao, *eLife*, 2019, **8**, e45833.
- 128 J. Crispell, S. Cassidy, K. Kenny, G. McGrath, S. Warde, H. Cameron, G. Rossi, T. Macwhite, P. C. L. White, S. Lycett, R. R. Kao, J. Moriarty and S. V. Gordon, *Microb. Genomics*, 2020, **6**, 1–8.
- 129 A. P. Ortiz, C. Perea, E. Davalos, E. F. Velázquez, K. S. González, E. R. Camacho, E. A. García Latorre, C. S. Lara, R. M. Salazar, D. M. Bravo, T. P. Stuber, T. C. Thacker and S. Robbe-Austerman, *Front. Vet. Sci.*, 2021, **8**, 674307.
- 130 C. Marianelli, B. Amato, M. B. Boniotti, M. Vitale, F. P. Ciarello, M. L. Pacciarini and V. D. M. Lo Presti, *PLoS Neglected Trop. Dis.*, 2019, **13**, e0007546.
- 131 P. Pozo, V. Lorente-Leal, S. Robbe-Austerman, J. Hicks, T. Stuber, J. Bezos, L. de Juan, J. L. Saez, B. Romero and J. Alvarez, *Front. Microbiol.*, 2022, **13**, 915843.
- 132 V. Valcheva, C. Perea, T. Savova-Lalkovska, A. Dimitrova, L. Radulski, I. Mokrousov, K. Marinov, H. Najdenski and M. Bonovska, *BMC Vet. Res.*, 2022, **18**, 1–12.
- 133 N. M. Zetola, P. K. Moonan, E. Click, J. E. Oeltmann, J. Basotli, X.-J. Wen, R. Boyd, J. L. Tobias, A. Finlay and C. Modongo, *Emerging Infect. Dis.*, 2021, **27**, 835.
- 134 M. De Garine-Wichatitsky, A. Caron, R. Kock, R. Tschoop, M. Munyeme, M. Hofmeyr and A. Michel, *Epidemiol. Infect.*, 2013, **141**, 1342–1356.
- 135 G. Almaw, A. Mihret, T. Abebe, G. Ameni, B. Gumi, A. Olani, M. Tamiru, T. Koran, A. Aliy, M. Sombo, S. Ayalew, A. Yesuf, H. Taye, J. L. N. Wood, S. Berg, A. Aseffa, B. Tessema, B. Belachew, E. Fekadu, F. Melese, G. Gemechu, R. Tschoop, S. Haile, T. Hailu, A. Bekele, C. Yirga, M. Ambaw, T. Mamo, T. Solomon, T. Teklewold, S. Gebre, G. Gari, M. Sahle, A. Sirak, G. Mekonnen, S. Guta, J. Wood, A. Conlan, A. Clarke, H. L. Moore, C. Hodge, R. G. Hewinson, M. Vordermeier, J. Nunez-Garcia, B. Bayissa, A. Zewude, A. Worku, L. Terfassa, M. Chanyalew, T. Mohammed and Y. Zeleke, *Zoonoses Public Health*, 2022, **69**, 663–672.
- 136 C. Perea, G. Ciaravino, T. Stuber, T. C. Thacker, S. Robbe-Austerman, A. Allepuz and B. P. de Val, *Microorganisms*, 2021, **9**, 1629.
- 137 A. Hauer, L. Michelet, T. Cochard, M. Branger, J. Nunez, M. L. Boschirololi and F. Biet, *Front. Microbiol.*, 2019, **10**, 955.
- 138 X. Jia, L. Yang, M. Dong, S. Chen, L. Lv, D. Cao, J. Fu, T. Yang, J. Zhang, X. Zhang, Y. Shang, G. Wang, Y. Sheng, H. Huang and F. Chen, *Front. Cell. Infect. Microbiol.*, 2017, **7**, 88.
- 139 G. Vernikos, D. Medini, D. R. Riley and H. Tettelin, *Curr. Opin. Microbiol.*, 2015, **23**, 148–154.
- 140 K. M. Ceres, M. J. Stanhope and Y. T. Gröhn, *Microb. Genomics*, 2022, **8**, 000839.
- 141 L. Muthukrishnan, *Chem.-Biol. Interact.*, 2021, **337**, 109397.
- 142 K. C. Januarie, O. V. Uhwo, E. Iwuoha and U. Feleni, *Anal. Bioanal. Chem.*, 2022, **414**, 907.
- 143 A. Rabi, A. Raouafi and N. Raouafi, *Nanotechnology Based Approaches for Tuberculosis Treatment*, 2020, pp. 207–230.
- 144 M. Gupta and L. S. Meena, *Journal of Nanotechnology and Nanomaterials*, 2020, **1**, 46–56.



- 145 P. K. Bairagi, A. Goyal and N. Verma, *Sens. Actuators, B*, 2019, **297**, 126754.
- 146 T. Pornprom, N. Phusi, P. Thongdee, B. Pakamwong, J. Sangswan, P. Kamsri, A. Punkvang, K. Suttisintong, J. Leanpolchareanchai and P. Hongmanee, *Talanta*, 2023, 125210.
- 147 E. Barbier, T. Fouchet, A. Hartmann, E. Cambau, F. Mougari, C. Dubois, M. Lubetzki and M. Rochelet, *Talanta*, 2023, **253**, 123927.
- 148 F. Mollarasouli, E. Zor, G. Ozcelikay and S. A. Ozkan, *Talanta*, 2021, **226**, 122108.
- 149 D. Örenli, C. K. Selvi, F. Öztürk, P. E. Erden and E. Kılıç, *Anal. Biochem.*, 2023, **662**, 115002.
- 150 X. Zhang, J. Zhu, Z. Wu, W. Wen, X. Zhang and S. Wang, *Anal. Chim. Acta*, 2023, **1239**, 340743.
- 151 G. Zhao, T. Pham, Y. Chen, M. Sedki and A. Mulchandani, *Microchem. J.*, 2023, **187**, 108376.
- 152 E. Torres-Chavolla and E. C. Alocilja, *Biosens. Bioelectron.*, 2011, **26**, 4614–4618.
- 153 X. Yu, Y. Chai, J. Jiang and H. Cui, *J. Photochem. Photobiol., A*, 2012, **241**, 45–51.
- 154 C. Wu, Y. Ma and L. Chen, *J. Electroanal. Chem.*, 2022, **907**, 116013.
- 155 N. K. Mogha, V. Sahu, R. K. Sharma and D. T. Masram, *J. Mater. Chem. B*, 2018, **6**, 5181–5187.
- 156 Y. Li, D. Peng, S. Guo, B. Yang, J. Zhou, J. Zhou, Q. Zhang and L. Bai, *Bioelectrochemistry*, 2022, **147**, 108209.
- 157 M. Zahran, Z. Khalifa, M. A. Zahran and M. A. Azzem, *Microchem. J.*, 2021, 106173.
- 158 M. Zahran, Z. Khalifa, M. A.-H. Zahran and M. Abdel Azzem, *ACS Appl. Nano Mater.*, 2020, **3**, 3868–3875.
- 159 M. Zahran, Z. Khalifa, M. A.-H. Zahran and M. A. Azzem, *Electrochim. Acta*, 2020, **356**, 136825.
- 160 L. T. Tufa, S. Oh, J. Kim, K.-J. Jeong, T. J. Park, H.-J. Kim and J. Lee, *Electrochim. Acta*, 2018, **290**, 369–377.
- 161 M. S. de Oliveira, E. A. de Oliveira Farias, A. M. S. de Sousa, N. A. Dionísio, P. R. S. Teixeira, A. S. d. N. M. Teixeira, D. A. da Silva and C. Eiras, *Surf. Interfaces*, 2021, **25**, 101245.
- 162 M. Shalauddin, S. Akhter, W. J. Basirun, M. Akhtaruzzaman, M. A. Mohammed, N. M. M. Abd Rahman and N. M. Salleh, *Surf. Interfaces*, 2022, **34**, 102385.
- 163 C. Guang-Lei, J.-C. Huang, Y. Jia-Qi, G. Ye-Min, L. Ming, Y.-Y. Zhang and S. Xia, *Chin. J. Anal. Chem.*, 2021, **49**, 1–9.
- 164 D. V. P. Thao, D. T. T. Ngan, D. Van Tuan, H. Lan, N. T. Nguyet, V. Van Thu, V.-P. Hung and P. D. Tam, *Mater. Today Commun.*, 2022, **33**, 104161.
- 165 Y. Liu, X. Yan, Y. Tang, N. Lu, T. Zhang, Z. Xu, Y. Xing, P. Zhao, M. Liu and Y. Zhu, *J. Electroanal. Chem.*, 2022, **926**, 116932.
- 166 A. K. Singh, *Curr. Res. Green Sustainable Chem.*, 2022, 100270.
- 167 D. Manoj, L. Gnanasekaran, S. Rajendran, A. Jalil, M. N. Siddiqui, F. Gracia and M. Soto-Moscoco, *Environ. Res.*, 2023, **222**, 115358.
- 168 R. Zokhtareh, M. Rahimnejad, G. Najafpour-Darzi and H. Karimi-Maleh, *Environ. Res.*, 2023, **216**, 114643.
- 169 M. Haddaoui, C. Sola, N. Raouafi and H. Korri-Youssoufi, *Biosens. Bioelectron.*, 2019, **128**, 76–82.
- 170 W. Jaroenram, J. Kampeera, N. Arunrut, C. Karuwan, A. Sappat, P. Khumwan, S. Jaitrong, K. Boonnak, T. Prammananan and A. Chaiprasert, *J. Pharm. Biomed. Anal.*, 2020, **186**, 113333.
- 171 S. R. Benjamin and E. J. M. R. Junior, *Current Opinion in Environmental Science & Health*, 2022, 100381.
- 172 Y. Hou, Y. Li, W. Huang, M. Tsunoda, Y. Zhang, X. Xie, R. Wang, L. Li, W. Hu and Y. Song, *J. Chromatogr. A*, 2020, **1632**, 461609.
- 173 C. P. McCarthy, K. M. Herdman, D. Rooney, B. Alcock-Earley and C. B. Breslin, *Synth. Met.*, 2021, **280**, 116881.
- 174 Y. Yan, P. Zhou, S. Zhang, X. Yin, X. Zeng, P. Pi, Y. Nong and X. Wen, *J. Water Proc. Eng.*, 2022, **49**, 102942.
- 175 R. Khoder and H. Korri-Youssoufi, *Mater. Sci. Eng. C*, 2020, **108**, 110371.
- 176 H. Ali, A. Yadav and N. Verma, *Chem. Eng. Process.*, 2022, **180**, 108656.
- 177 K. S. Rizi, B. Hatamluyi, M. Rezayi, Z. Meshkat, M. Sankian, K. Ghazvini, H. Farsiani and E. Aryan, *Talanta*, 2021, **226**, 122099.

