**NANOTECHNOLOGY APPROACHES FOR AMR DIAGNOSTICS**

-rapid detection and reliable

-high sensitivity

-less resource-intensive (instrument-free)

-more affordable

-simpler methodologies - semiskilled user

-overall, numerous improvements in AMR diagnostics over classical approaches with respect to test duration, precision, accuracy, sensitivity, range of biological samples, simultaneously test large sample numbers, portability and cost-effectiveness

-classical approaches sometimes multiple days to produce results, nanotechnology within few minutes

-test multiple samples simultaneously

-less or no requirement for heavy instrumentation, more affordable

-cost can be reduced by incorporating mass manufacturing approaches

-can measure detection range sufficient to determine level of resistance or infection

-more portable, conveniently taken to different regions and rural areas

-follow WHO’s guidelines

-extensive research, but not yet to market globally: regulatory concerns, poor awareness about AMR among general public, etc.

**4.1. Nanomaterial-Based Nanosensors**

-nanomaterials, such as metallic nanoparticles, quantum dots, fluorescent nanoparticles, magnetic nanoparticles, upconversion nanoparticles, and carbon based nanoparticles

-without requiring any specific bioprobe

-high surface-to-volume ratio and tunable surface properties

-reliable results, but requirement of high-end sophisticated instruments like MRI (Magnetic Resonance Imaging), NMR (Nuclear Magnetic Resonance) and fluorescence spectroscopy for analysis, combined with complexity of data interpretation, limit application to laboratory use mostly.

-due to high surface-area-to-volume ratio, nanomaterials can hold large number of biomolecules on surface, such as antibodies, which can increase detection sensitivity of the device

-the other nanomaterials have limitations compared to metallic nanoparticles, such as difficult synthesis, requiring instrumentation like fluorescence microscopes or spectrophotometers (for detection) and high toxicity in the case of QDs, makes metallic nanoparticles valuable in fashion similar to pregnancy tests; most other nanomaterial-based assays also need use of metallic nanoparticles as a reporter molecules. Apart from this, all nanomaterials are useful based on the required applications, such as MNPs used to reduce high interference when testing AMR in complex biological samples and fluorescence related nanomaterials and carbon nanomaterials used to improve sensitivity with minimal instrumentation.

**4.1.1. Metallic Nanoparticles**

Gold nanoparticles (AuNPs)

-most popularly used

-size and shape-dependent distinct optical properties compared to bulk materials

-variation of size, shape and interparticle distance, optical properties modulated, causes change in color

-work even without requirement for instrumentation

-can give results in the form of color change, easier to interpret, similar to pregnancy tests

**4.1.2. Quantum Dots and Fluorescent Nanoparticles**

-Quantum dots (QDs) group of synthetic inorganic semiconducting materials

-size of few nm

-distinct optoelectronic properties

-emit light of specific wavelength when current is applied or when exposed to light

-precise control over their emission wavelength by varying physical dimensions or excitation wavelength or by chemical modification of their surface to be able to visualize the results with naked eyes

-better than fluorescent dyes due to their unique optical properties

**4.1.3. Magnetic Nanoparticles**

-separate bacterial content from complex biological samples like blood and sputum and carry out their affinity-based purification when placed under a strong magnetic field

-high-sensitivity MRI-based detection tools for pathogenic infections

-reactive surfaces, often functionalized with suitable ligands to selectively bind to the target pathogen, notable change in relaxation time or alteration in the magnitude of magnetic properties

-improve signal-to-noise ratio by separating molecules to be detected from nonspecific molecules thus reducing background signal

**4.1.4. Upconversion Nanoparticles**

-luminescent nanomaterials

-distinct optoelectronic properties

-2 or more low-energy photons absorbed and upconverted to single and sharp high-energy photon emission, depending on wavelength

-excellent photostability, sharp emission bands and tunable surface properties

-can help in obtaining high sensitivity due to unique anti-Stokes shift

**4.1.5. Carbon-Based Nanomaterials**

-polymeric nanoparticles, lipid-based nanoparticles and carbon nanotubes (CNTs) are some popular examples;

-unlike inorganic nanoparticles, very high surface-to-volume ratio due to nano- or micro-porous architecture, allows easy encapsulation of molecules required for pathogen detection;

-diverse functional groups in their chemical structure

-large surface area for biomolecule attachment due to porous architecture

**4.1.6. Nanoparticle-Based Point-of-Care (POC) Rapid Diagnostics**

-for rapid AMR detection, even in low-resource settings

-should essentially comply with the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, deliverable to end-user) guidelines issued by WHO

-seem to be most appropriate set of devices for immediate deployment and use in diagnostic schemes as the ASSURED and instrument-free advantages make them leading candidates

**4.2. Nanoparticle-Based Biosensors**

-capable of measuring different parameters (analytes) and convert information into signal that can be read or analyzed

-main focus results can be visualized with naked eyes or using minimal instrumentation like portable mobile phone-based applications.

**4.2.1. Optical Biosensors**

-measure interaction of analyte with photons

-colorimetric biosensors primarily leverage color changing property of nanoparticles

-optical biosensors require minimal instrumentation for visualization

**4.2.2. Mechanical Biosensors**

-also known as **cantilever sensors** because utilize atomic force microscope (AFM) probe or cantilever for pathogenic detection

-shape of probes or cantilevers varies from rectangular to triangular, long enough to bend and give small oscillations (on the order of 1−100 nm) when encounter analyte

-deflections in probe (generated as a consequence of interacting with the analyte) measured by laser beam technique

-deflections generated due to interaction of probe with analytes continuously recorded and interpreted to distinguish between resistant and susceptible bacteria

-greater deflection signifies presence of resistant bacteria as they survive in the presence of antibiotics

**4.2.3. Electrochemical Biosensors**

-based on measurement of changes in current flowing through electrochemical cell upon changing applied voltage to electrode system or monitoring current with respect to time

-signal generated based on intensity of redox reactions (oxidation/reduction of redox-active species) inside bacteria, which vary with respect to growth kinetics and viability of bacterial cells in response to antibiotic treatment

-depending on variable susceptibility of bacteria to different antibiotics, growth behavior changes, reflected in change in redox-reactions, leading to changes in detected voltage

**4.2.4. Microfluidic-Integrated Biosensors**

-fluids in very small volumes ranging from few mL to nL and sometimes even pL scale. Inside channel, unique phenomena such as laminar flow and differential mixing, not observed at a larger volume scale.

-Depending upon viscosity, ionic concentration, temperature, pressure and other hydrodynamic variables, different fluids with different laminar flow and intermixing properties

-unique advantages of using lowest sample volume, less reagent, minimal incubation time and potentially generating rapid results

-nucleic acid-based detection involves culturing of bacteria or extraction of genomic DNA, which is time consuming and cumbersome, making this process less preferable

**4.2.5. Other Nanoscale Biosensing Techniques**

-field-effect transistors (FETs), nanowires, quartz crystal microbalance (QCM), thin film transistor (TFT) nanoribbons and chemical tongue chips (CTCs) for AMR detection