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Smartphone based dual mode *in situ* detection of viability of bacteria using Ag nanorods array

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ABSTRACT

The *in-situ* and rapid detection of live and dead bacteria is essential for human and environmental care. It has become one of the biggest needs in the biological and medical sciences to prevent infectious diseases, which usually occur in hospitals and field clinics. In the current scenario, antibiotic resistance is one of the severe public health problems, which requires a quick and efficient solution. Here, we report a facile sensitive, portable, user-friendly, cost-effective and time saving approach for detection of live, dead and drug-resistant bacteria. The endogenous H_2S evolution was targeted to differentiate between live and dead as well as antibiotic resistant bacteria. The silver nanorods (AgNRs) arrays sensors were fabricated by glancing angle deposition technique. The colorimetric and water wettability features of as-synthesized AgNRs are found to be highly sensitive and selective for H_2S. *E. coli. P. aeruginosa, B. subtilis and S. aureus* were used as a model organism in this study. All the bacteria were found to produce H_2S by their metabolism process. In order to detect the antibiotic resistant *E. coli* were grown in the presence of different concentration of ampicillin in Luria broth. A drastic visible change in color as well as wetting of AgNRs array was observed. To make the technique easy, a user-friendly and field deployable mobile app 'Colorimetric Detector' was developed. This technique takes only 4–6 h whereas the conventional methods need around 24 h for the same. This dual mode facile and, inexpensive method can be easily scaled up in the field of diagnostics.

1. Introduction

According to world health organization (WHO) report 2018, increasing antibiotic resistance (ABR) of microbes has raised global concern for human health and is currently a major threat to control the infectious diseases. The effect of treatment of patients with antibiotics often leads to the evolution of antibiotic resistance in the pathogens. When a person takes the antibiotic drug, almost all bacteria killed by it, leaving behind a few numbers of resistant microbes, which grow even in the presence of the antibiotic. Overuse or misuse of antibiotics is a major modifiable cause of an increase in drug-resistance in bacteria. When antibiotics do not kill bacteria, infections often last longer, cause more severe illness, longer hospital stays, and involve more expensive and toxic medications. Sometimes infections by resistant bacteria become so threatening that can even cause death. The increasing number of pathogens and incidence of ABR in patients are of great concern for the treatment of pathogenic diseases (Alswat et al., 2016; Ullah et al., 2018). On the other hand, the pathogens dwelling on edible products are also of great concern for the health and well-being of human and other living creatures, hence the estimation of live and resistant microbes is crucial in the hospitals, field clinics, dairy products, potable water system (LeChevallier et al., 1980) sewage management system and so on (Li et al., 2018). In addition, *in situ* measurement of bacterial cells during antibiotic treatment regimen may help in the assessment of efficacy of treatment. Thus, the differentiation of live and dead as well as antibiotic resistant microorganisms is of great importance.

The most commonly used and accepted method for the estimation of live bacteria is the standard plate count (SPC) method (Ericsson et al., 2000). However, it is time consuming and highly prone to manual errors (Brugger et al., 2012). This method is also costly due to a large number of culture plates and a high amount of media required during the whole process. Therefore, researchers generally use alternative methods such as automated counting of bacterial colony forming units (Brugger et al., 2012), fluorescence microscopy (Johnson and Criss, 2013) for routine measurements. These methods also require high skill and sophisticated instruments, which restrict their usage. On the other hand, UV–vis spectroscopy is another most commonly used alternative method to estimate the number of bacteria per unit volume of the

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sample but lack the information whether the bacteria are alive or dead (Koch, 1968). Although many methods are available for bacterial estimation, more recently, *in situ* method for detection of live/dead bacterium using synchronous fluorescence and principal component analysis (PCA) have been developed (Li et al., 2018), nevertheless, a relatively facile, fast, sensitive, selective, reproducible, user-friendly and cost-effective method development is needed. Here, we have demonstrated a new easy and inexpensive method which overcomes all the challenges occurred in aforementioned conventional methods.

Hydrogen sulfide (H_2S) was arguably the most versatile molecule when life began; it could serve as an important organic molecule, reactant, catalyst (proto-enzyme), barrier (proto-membrane), and sustainable source of energy (Olson and Straub, 2016). The H_2S emitted from living organisms (biogenesis) is primarily due to the metabolism of sulfur-containing amino acids *viz.* cysteine and methionine of protein. Several species of bacteria and many types of animal cells have well-developed pathway for H_2S biogenesis. H_2S is also known to be the latest member of physiologic gasotransmitter family with nitric oxide (NO) and carbon mono oxide (CO) that transmit the signals in mammalians cells. *E. coli* carries mercaptopyruvate sulfurtransferase (MST) which produces H_2S endogenously from cysteine during exponential growth of bacteria in nutrient rich media (Shatalin et al., 2011). MST is involved in the conversion of 3-mercaptopyruvate derived from cysteine transamination to pyruvate (Filipovic et al., 2017; Kabil and Banerjee, 2014).

Orlowski was first to observe the emission of H_2S by the bacteria in 1895 and since then, the researchers have used it as a tool for various diagnostic purposes such as to distinguish between the paratyphoid and enteritis group of bacteria (Clarke, 1953). There are few methods, which have been used by previous researchers for the determination of H_2S production by bacteria. It includes lead acetate, mercuric paper test, and silver nanoparticle supplemented media (Carpenter et al., 2017; Jarosz et al., 2013). Apart from these, bismuth-based reflectance from silver plate, sodium nitroprusside and methylene blue wet chemical methods, infrared spectrophotometry and gas chromatography have also been explored in order to detect H_2S. (Liu et al., 2018; Vitvitsky and Banerjee, 2015; Xiong et al., 2013; Zeng et al., 2016; Zhao et al., 2017). Among them, the lead acetate test strips based method has been used most commonly as a sensor of H_2S emitted from bacterial culture. These white strips turn black upon the formation of black colored lead sulfide but this lead-based method has some drawbacks as it is highly toxic and less sensitive (Lawrence et al., 2000).

In this study, the experiments were carried out with four bacteria: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 14886, *B. subtilis* ATCC 6633 *and S. aureus* ATCC 9144 purchased from the American Type Culture Collection (USA) for determining the viable (live) and nonviable (dead) bacteria in a sample using colorimetric and wettability of silver nanorods (AgNRs) array fabricated by glancing angle deposition (GLAD) technique. Although these four microbes were found to produce H_2S and exhibit the change in color as well as wettability, *E. coli* were taken for detailed study. *E. coli* are numerous in the environment, foods, as well as in the intestines of animals including humans. Although most of the strains of *E. coli* are harmless, some strains are highly pathogenic and cause diseases like diarrhea, acute kidney failure, hemolytic-uraemic syndrome (HUS) and hemorrhagic colitis *etc.* (Cho et al., 2015; Güner et al., 2017; Kaper et al., 2004). Pathogenic strains of *E. coli* are clinically relevant; it is usually present in the unprocessed food, raw milk, contaminated water, and vegetables. Data obtained in the present study show the potency of the system to identify live/dead bacteria with or without antibiotic treatment. The conventional methods *e.g.* standard plate count needs around 2–3 days whereas this facile method takes only 4–6 h to determine the viability and resistance microbes. Therefore, it is a fast, simple and cost-effective method for the estimation of live and resistant microorganisms. The system relies on the ability of most of the living microorganisms to produce H_2S gas during metabolism and the detection of this gas using AgNRs array. However, the amount of production varies from species to species (Clarke, 1953; Kolluru et al., 2013). Interestingly this method has potential to be used as a tool for *in situ* detection of live and antibiotic resistant bacteria in the wound and thus could help in treatment as well as monitor the presence of viable pathogens present in packaged food products.

2. Materials and methods

2.1. Synthesis of AgNRs array

For the detection of H_2S gas from bacteria, the AgNRs arrays were grown on glass substrates by the physical evaporation method, using the GLAD technique. The glass substrates were washed with ethanol (70%), followed by piranha solution (4:1 sulfuric acid, hydrogen peroxide). Then the substrates were rinsed with deionized water followed by drying under the stream of nitrogen gas before loading into the deposition chamber. The substrates were mounted on a sample holder such that the angle between the substrate normal and the incident vapor flux was 85°(Gahlaut et al., 2017; Karabacak et al., 2003; Kumar et al., 2014; Mark et al., 2013; D. P. Singh et al., 2012; Zhao et al., 2002). The highly pure (99.9%) 2 g of silver metal powder was thermally evaporated in high vacuum (2×10^{-6} Torr) chamber. During the initial growth, the impinging silver atoms form isolated nucleation centers which cast shadows for the arriving vapor flux. The nucleated islands act as shadowing centers; therefore, the larger nucleation centers will receive more impinging atoms as compared with the smaller ones, and only the larger islands will grow. The competition between limited adatom surface mobility and shadowing effect results in the evolution of the rod shaped columnar structure, with the growth of AgNRs in the direction of the incident vapor flux (Karabacak et al., 2003; D. P. Singh et al., 2012; J. P. Singh et al., 2012).

2.2. Culture conditions for growth of bacterial strains

The *E. coli* ATCC 25922, *P. aeruginosa* ATCC 14886, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 9144 purchased from the American Type Culture Collection (USA) were grown in the Luria broth (LB) culture medium. The 10 ml of the media was prepared in 50 ml Erlenmeyer flask by dissolving 0.2 g Luria broth powder (Himedia, India) in deionized water. The media was autoclaved at 15 psi pressure and 121 °C temperature for 15 min. To make the culture antibiotic resistance, the *E. coli* bacterial culture was transfected with pUC19 plasmid vector having ampicillin resistant gene in it. The transfected culture was screened using ampicillin supplemented media.

Before the sensing experiment, the bacterial strains were subcultured in the Erlenmeyer flask containing LB media with and without ampicillin supplementation. The normal and antibiotics resistant *E. coli* were grown in an incubator shaker at 37 °C at 200 rpm. In exponential phase number of bacterial cells were determined after calibration, where 0.1 optical density (OD) at 600 nm corresponds to 10^8 cells/ml. Based on this, 10^2 bacterial cells were inoculated into the broth culture supplemented with 0, 25, 50 and 100 μg/ml of ampicillin for experiments. The ampicillin, as indicated in the experiment was added to the media after filter sterilization with 0.2 μm sterilized nylon membrane filter. The minimal bactericidal concentration (MBC) for this antibiotic towards *E. coli* (NEB5α) was found as 100 μg/ml.

For gas sensing experiments, 10 μl culture suspension was inoculated in the Luria broth with and without ampicillin, as described above. The as grown AgNRs arrays on glass (1.50×1.25 cm^2) were mounted horizontally facing down, at the neck of each Erlenmeyer flask and capped properly in the laminar airflow hood to ensure proper aseptic condition. The cultures were then incubated at 37 °C at 200 rpm at the orbital shaker for optimal growth. After 16 h of incubation, the AgNRs substrates were taken out to characterize.

2.3. Fluorescence imaging

E. coli were visualized by live/dead bacterial staining using acridine orange and ethidium bromide (Goel and Mishra, 2018; Jaiswal and Mishra, 2018). The *E. coli* were stained with a mixture of acridine orange (100 mg/L) and ethidium bromide (100 mg/L) in the dark. After staining, the *E. coli* cells were washed twice by phosphate buffered saline (PBS) (pH 7.2) to minimize background fluorescence. Finally, the *E. coli* cells were observed under IX51 inverted fluorescence microscope (Olympus Inc., Japan). Images were captured using Olympus DP70 camera at a magnification of 20 × .

2.4. Wettability based detection

The wettability of the substrates was measured in terms of the contact angle (CA) of sessile drop kept on the AgNRs array. The CAs were measured before and after the experiment by Kruss drop analyzer 25 DSA (KRÜSS GmbH – Germany). For this, 2 µl of deionized water was dropped by an automated syringe attached to the instrument.

2.5. Mobile app development for colorimetric detection

We have developed an android based mobile app with an attachment for the colorimetric detection. One can attach it in front of the mobile camera and the AgNRs array is placed in the direction perpendicular to the optical axis of the camera. In order to have clear visibility of the image and constant illumination, we have used LED light. For a user-friendly portable module, the android app "Colorimetric Detector (CD)" was developed in the JAVA platform. This app was successfully installed and tested in android version 6.0 smart-phones. The relative change in intensity is referred by the term optical darkness ratio (ODR) (Gupta et al., 2007) which is defined as the relative change in grey scale intensities of the reflected light with respect to the background.

$$ODR = \frac{I_b - I_s}{I_b}$$

Where, I_b and I_s are grey scale intensity of pristine (background) and exposed AgNRs array (substrate), respectively.

Thus, the value of ODR is given by the app and verified with the MATLAB code. A demonstration of the app has been shown in Fig. S1. This user-friendly portable device directly calculates and compares the ODR values. When it detects H_2S gas above the set point value, the mobile phone vibrates and gives a red signal. Lower than the set point value ODR shows green signal.

3. Results and discussion

To detect the evolution of H_2S from bacterial culture, AgNRs array bio-sensors were fabricated on glass substrates by GLAD method. This method is well known for the fabrication of highly pure and uniform arrays AgNRs (Karabacak et al., 2003). The nanorods having a high surface area in comparison to conventional thin film, greatly enhance the sensitivity of the gas detection. The substrates were characterized, before and after the H_2S gas sensing experiments. The scanning electron microscope (SEM) [model ZEISS EVO 50] was used for the morphological confirmation. The average length and diameter of Ag nanorods were found to be 1 µm and 150 nm, respectively (Fig. 1(a)). The inter-rod separation was found to be about 50 nm. The slanted nanorods were uniformly grown on the glass substrates (1.50 cm × 1.25 cm), which imparts it a whitish texture, as shown in the inset of Fig. 1(a). Atomic force microscopy (AFM) (model Dimension Icon, Bruker, USA) was used to investigate the morphology of the AgNRs substrates. The images were collected in tapping mode® with probe Tap 190, which has a nominal spring constant value of 40 N/m. The images were processed and analyzed with the 'Nanoscope' software. The histogram shown in Fig. 1(b) inset depicts the frequency distribution "percentage of observation" of the diameter of nanorods. It shows the monodispersed rods with average diameter 150 nm.

The silver peak in the spectra of energy dispersive X-ray (EDX) spectroscopy in Fig. 1(c) shows the presence of silver metal without any impurity. The peaks of other elements *i.e.* calcium, and silicon are due to the glass substrates on which the nanorods were grown. The X-ray diffraction (XRD) spectra in Fig. 1(d) shows the pure crystalline phase of pristine AgNRs (JCPDS 04–0783). For sensing H_2S gas emission from bacterial culture, the pristine substrates were mounted horizontally at the neck of each Erlenmeyer flask, facing downward as shown in Fig. 2(a). The AgNRs substrates were exposed to the H_2S gas for different time duration of 4, 8, 12 and 16 h, respectively.

The EDX of an exposed array, as shown in Fig. 2(b) confirms the presence of sulfur in *E.coli* exposed arrays. The H_2S emitted from the bacterial culture reacts with silver to form silver sulfide (Ag_2S) through the process of sulfurization at ambient condition. To determine the crystal phase of Ag_2S, the XRD spectra were taken on the exposed array. The XRD spectra of pristine samples were matched with silver whereas the exposed spectra were matched with that of silver sulfide (JCPDS# 14-0072). It confirms the formation of the standard monoclinic phase of α-Ag_2S (acanthite) as shown in Fig. 2(c). To determine the presence of live bacteria, we relied on the change in color as well as the wetting property of the AgNRs array. After 6 h of exposure of AgNRs arrays to the bacterial culture, a noticeable change in color and wetting properties of the array was observed. However, substrates taken at the end of 16th hour shows the comparatively significant change in color compared to the unexposed AgNRs substrates. This change in color was measured in terms of ODR. The ODR value was measured by a mobile phone with an in-house developed android app "Colorimetric Detector". This method is self-sufficient to distinguish between live and dead cells. Along with it, a unique approach has also been proposed based on the water wetting property of AgNRs array. The wettability of the AgNRs array was determined by measuring water contact angle using Kruss drop shape analyzer DSA 25 Expert. This might be the other potential method for determination of viability in a microorganism.

From these results, it is clear that AgNRs sensors are sensitive enough to detect the H_2S emitted from the live bacterial culture within 6 h. At this stage, the bacterial culture is in log phase, in which the number of live cells increases exponentially and the culture is in metabolically most active phase. The percentage change in the ODR and CA values increase with an increase in time. But, 6 h time is enough to distinguish between live and dead bacteria.

Based on more than 100 observations, we have determined the threshold value to distinguish live and dead bacteria. The threshold values for change in CA and ODR are 10% and 18%, respectively. Only above the threshold value, the bacteria were found to be alive. It is depicted by a demarcation line in the Fig. 3(b).

Most of the living organisms produce several gases and volatile organic compounds (VOC) during their metabolism which have been studied earlier (Beauchamp et al., 1984; Kolluru et al., 2013; Vandiver and Snyder, 2012). H_2S is known to be one of the main products. There are mainly three enzymes involved in metabolism CBS, CSE, MST which are known to be responsible for endogenous H_2S production in all organisms. The *E. coli* carries MST enzyme which is found to be responsible for the emission of H_2S (Shatalin et al., 2011). It is important to notice that only metabolically active live cells are able to emit the H_2S. Therefore, it has been used as a biomarker for the live cell.

The determination of antibiotic-resistant bacteria has been done at a minimum bactericidal concentration (MBC) where all bacteria are killed; no change in color, as well as contact angle. was noticed which is equivalent to the control sample. The effect of concentration of antibiotic Ampicillin on colorimetric and wetting properties of AgNRs substrates was studied and results are shown in Fig. 4. The change in color of AgNRs substrates is quite evident which is conferred by the mechanism of sliver sulfidation. The variation in color and wetting behavior of the AgNRs array may be attributed to the conversion of

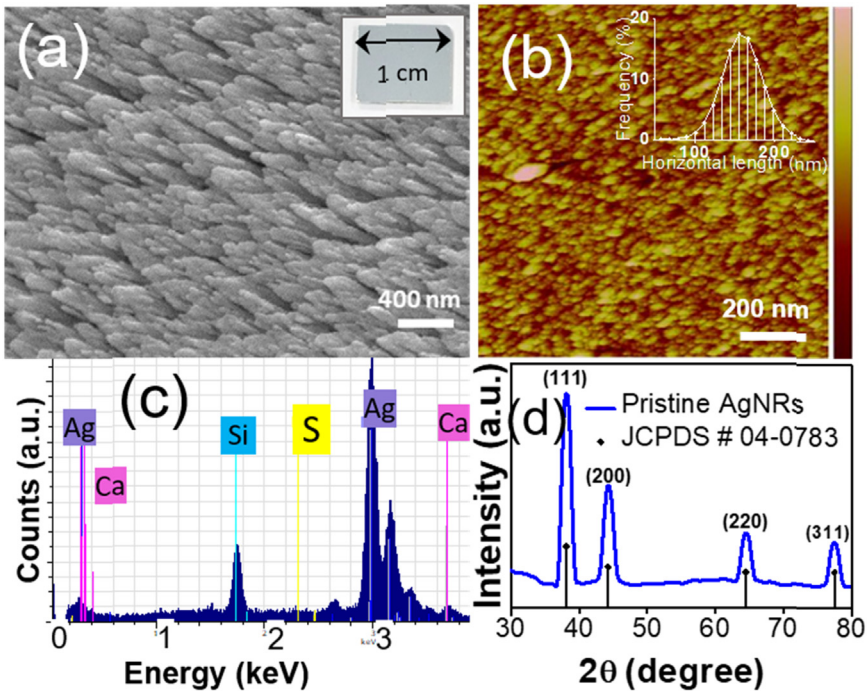

Fig. 1. The characterization of pristine AgNRs array. (a) The scanning electron micrograph (SEM) image taken at 30KX magnification showing uniform slanted silver nanorod array. The photograph of glass substrate on which the array were grown is depicted in the inset. (b) AFM image of AgNRs array showing morphology and the size distribution histogram of nanorods is shown in the inset. (c) EDX spectra of pristine nanorods array showing the presence of Ag. The calcium, and silicon peaks are present from the glass substrate. The absence of S depicts that the substrates are pure before any exposure. (d) XRD spectra of pristine AgNRs array matching with JCPDS.

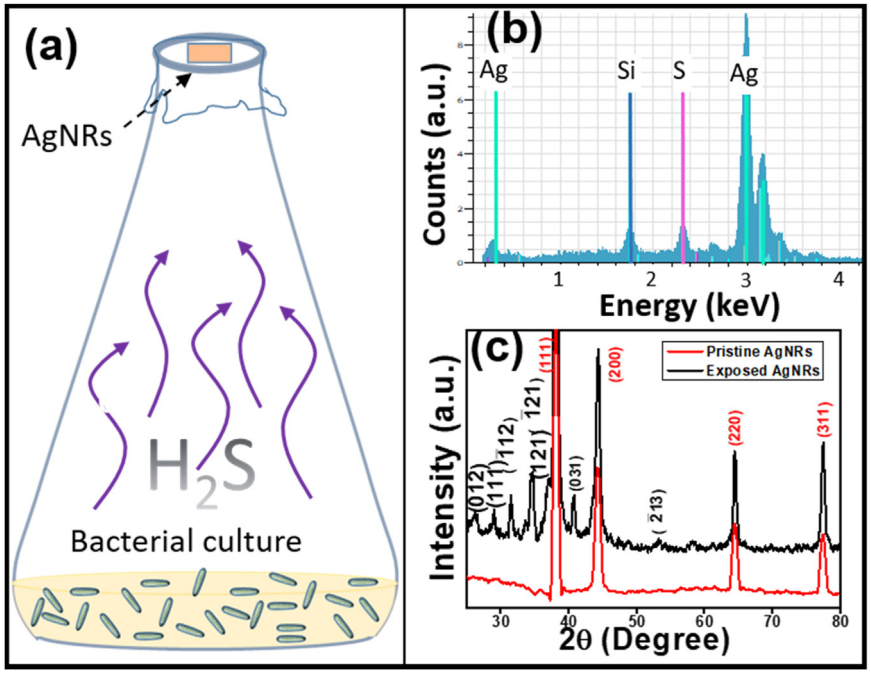

Fig. 2. (a) The schematic depicts the mounting of the AgNRs array on Erlenmeyer flask. (b) The EDX of exposed sample. (c) The XRD spectra of exposed and pristine AgNRs array.

silver to silver sulfide (Ag_2S) on the surface of array. The conversion can be easily described by the following reactions.

$$H_2S \rightarrow HS^- + H^+ \quad (1)$$

$$8Ag + 4HS^- \rightarrow 4Ag_2S + 2H_2 + 4e^- \quad (2)$$

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (3)$$

At ambient temperature, the gaseous H_2S molecules dissociate and become available for reaction with silver to form blackish silver sulfide as shown in Eqs. (1) and (2). The presence of hydroxyl ions as the product of the reaction (Eq. (3)) leads into water adsorption on the AgNRs array surface that results to decrease in water contact angle (Gahlaut et al., 2017).

The color of the sulfide layer depends on its thickness on the silver surface. Due to the reflection by the Ag_2S thin film, reddish, yellowish, and bluish colors were observed as shown in Fig. 4. However, for simplicity, we have used grey scale values only. To rule out the effect of culture medium, we have also performed a control experiment in which medium Luria broth was taken without bacteria. Interestingly, it neither changes AgNRs color nor the water contact angle value of the AgNRs arrays.

It is evident from Fig. 4 that the sensing parameters *viz.* color and

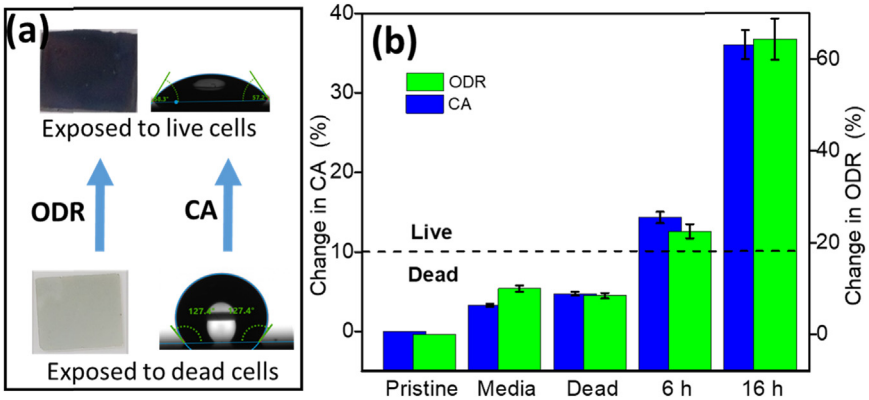

Fig. 3. The determination of live and dead bacteria by change in ODR and CA (a) the photograph of sensing array and water droplet placed on the array for the ODR and CA measurement respectively. (b) The graph showing percentage change in ODR and CA before (pristine) and after 6 h and 16 h of culture. The media and heat-killed bacteria were taken as control. We have drawn a demarcation line, which represents the threshold values above which the live bacteria is distinguishable from dead bacteria.

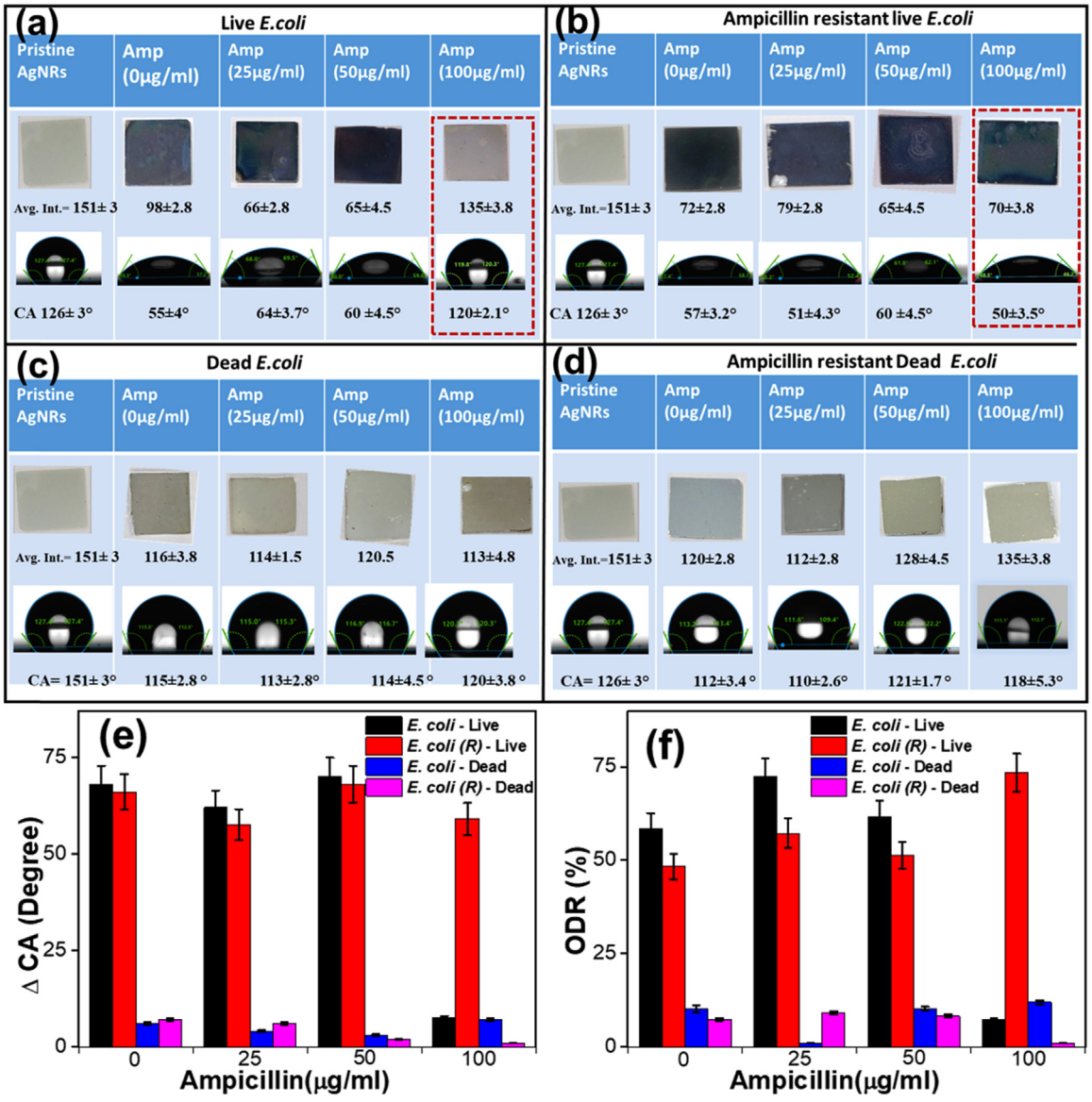

Fig. 4. Depiction of images showing the change in color and wettability (contact angle) of AgNRs array at different concentration of ampicillin for (a) Live *E. coli*, (c) Dead *E. coli,* (b) Ampicillin resistant live *E. coli* and (d) ampicillin resistant dead *E. coli.* The red rectangles indicate the difference in normal and ampicillin resistant *E.coli.* The change in (e) contact angle (CA), (f) Optical Darkness Ratio (ODR) of the AgNRs array after exposure the normal and ampicillin resistant *E. coli* culture supplemented with different concentrations of ampicillin. The heat killed (dead) bacterial culture was used as a control. All the photographs images have resolution of 4096 × 3072 with 12.6 megapixels mobile camera.

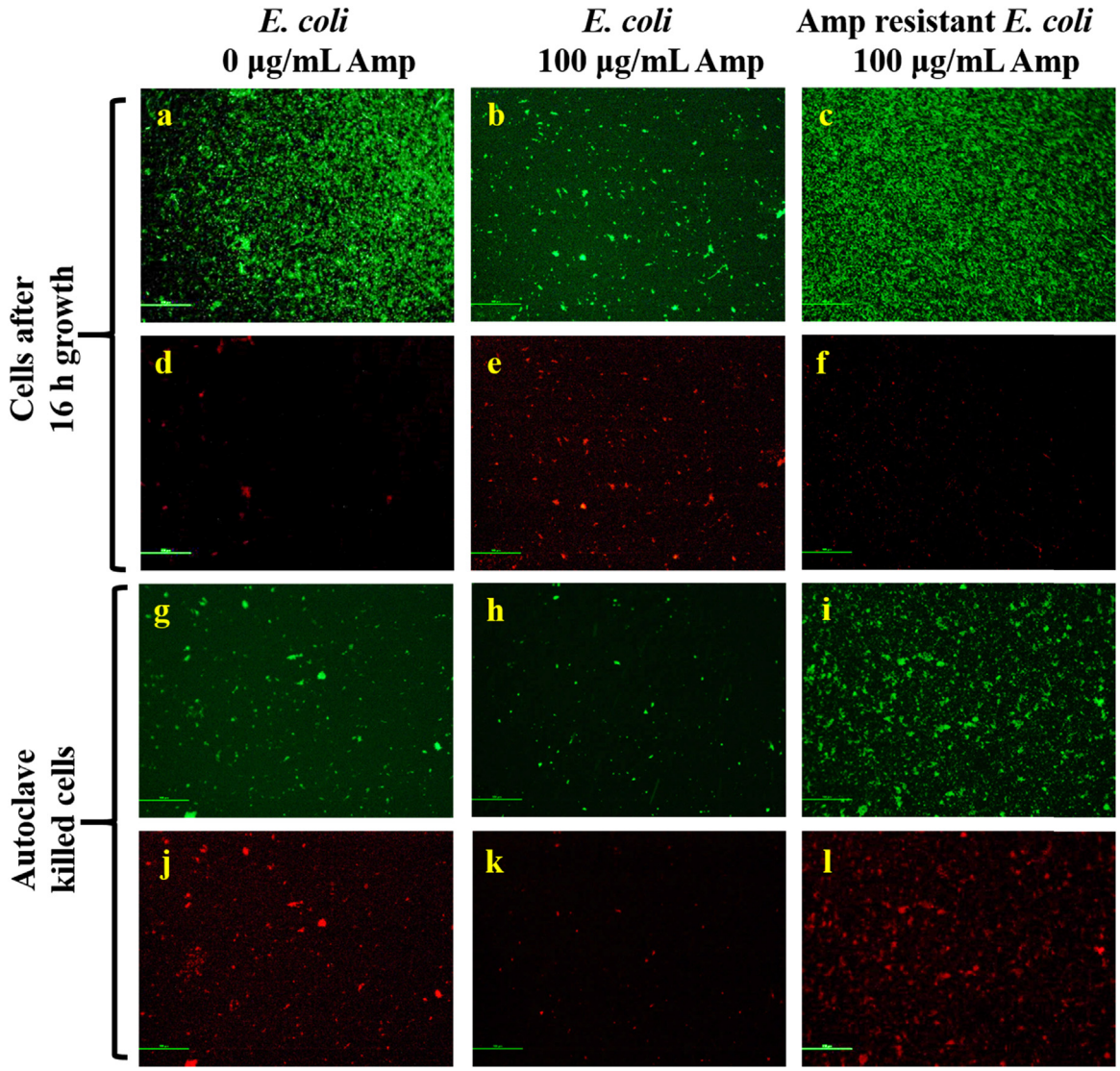

Fig. 5. Fluorescence imaging of *E. coli* and ampicillin resistant *E. coli* grown in Luria broth for 16 h. In fluorescence image, green represents acridine orange and red represents ethidium bromide: Images of *E. coli* grown in the absence and presence of ampicillin in (a, d), (b, e) respectively and ampicillin resistant *E. coli* (c, f). The *E. coli* were killed by autoclaving at 121 °C and 15 psi. The imaging of *E. coli* (g, h, j, k) and ampicillin resistant *E. coli* (i, l) after autoclaving are shown. The scale bar is equal to 100 nm in each image.

wettability were drastically changed from pristine to *E.coli* exposed AgNRs array. In live bacteria up to ampicillin concentration value of 50 µg/ml, the change is almost constant. But at ampicillin concentration 100 µg/ml at which all the *E. coli* were killed, no change in color as well as wettability of AgNRs array was observed (Fig. 4(a)). This concentration (100 µg/ml) is therefore regarded as MBC for these bacteria. The standard protocol given by Clinical and Laboratory Standard Institute USA was followed for determination of MBC (Barry et al., 1999). When the culture containing antibiotic resistance gene (Fig. 4(c)) compared with that of their normal counterpart (Fig. 4(a)) at ampicillin concentration value of 100 µg/ml, it can easily be distinguished (shown in red rectangles). The antibiotic resistant culture is still live and produces H_2S at MBC whereas normal bacteria do not. Neither dead bacteria nor culture media alone produce H_2S.

The graphical representations of the ODR and CA values are shown in Fig. 4(e) and (f). The changes in CA and ODR values of live cells were more than 50%, which confirms the reliability of this detection system for the determination of live, dead and antibiotic-resistant bacteria.

We have verified our results with that of existing alternative methods *viz.* fluorescence microscopy (Fig. 5) and CFU counting

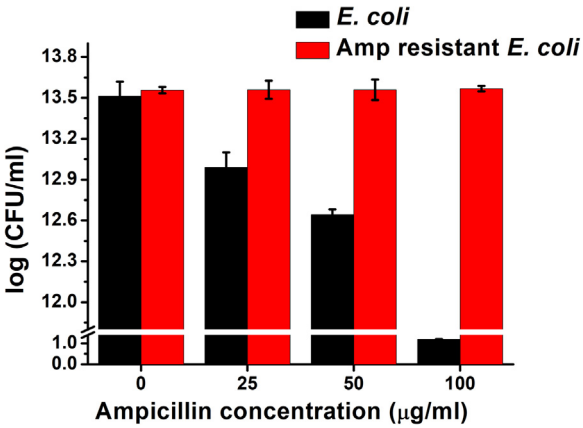

Fig. 6. The SPC method depicting effect of ampicillin concentration on number of CFU. At MBC (100 µg/ml), no growth of normal *E.coli* was observed.

method (Fig. 6). In the fluorescence image, green fluorescence represents cells (live and dead both), whereas the dead cells are represented by red fluorescence. The number of dead bacteria (Fig. 5(g–l)) was very few compared to live (Fig. 5(a–f)), and as the antibiotic concentration increased to 100 μg/ml (MBC), the number of dead bacteria increases enormously in case of normal *E. coli* (Fig. 5(e)) whereas in case of resistant *E.coli* only a few were dead (Fig. 5(b)).

The effect of ampicillin on the antibiotic resistant and non-resistant bacteria was determined using the SPC method and results are depicted in Fig. 6. The numbers of live non-resistant bacteria decrease due to supplementation of ampicillin in the culture media whereas there is an almost negligible effect of ampicillin on ampicillin resistant bacteria.

From these results, it is clear that the live *E. coli* produces H_2S gas, which can be used to distinguish live and dead bacteria within 6 h as compared to other conventional methods such as SPC which takes about 16–24 h. Thus, the proposed method meets all the requirements for a biosensor which renders a portable, user-friendly, selective, simple and fast method to distinguish live/dead as well as antibiotic resistant bacteria and may be used for *in situ* monitoring of the efficacy of antibiotics which makes this method superior to other existing techniques.

4. Conclusion

We have successfully demonstrated a rapid, cost-effective, portable, single step method to determine live and dead microbes. The laboratory strains of *E. coli, P. aeruginosa, B. subtilis and S. aureus* were used for the present study. The endogenous gas (H_2S) detection from bacteria was employed to determine their viability. For the detection, colorimetry and water wetting properties of silver nanorods array fabricated by GLAD were utilized. The color and water wettability of as grown AgNRs were found to be highly sensitive toward the H_2S gas evolved from the bacteria. The change in color, as well as wetting behavior has been described in terms of ODR and contact angle respectively. The change in these parameters has been observed for the live bacteria while autoclaved killed bacteria do not show any such change. The variation in color of AgNRs arrays was measured by an in house developed mobile app 'Colorimetric Detector'. It is clear that the detection of H_2S gas emitted from bacteria can be used to distinguish live and dead bacteria within 6 h, which is found to be better than conventional SPC method that takes about 16–24 h. The results have been also verified by conventional methods *e.g.* CFU and fluorescence imaging methods. This viability test approach based on gas detection may have possibility in future to determine resistance in microbes. The present approach has potential application in hospitals and clinics for the diagnosis of infections and diseases caused by antibiotic resistant and non-resistant pathogens.

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Competing interest

The authors declare no competing financial interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.11.025.

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