

Detection of Pathogenic Bacteria in Aqueous Media: Assessing the Potential of Real-Time Electromagnetic Wave Sensing

I. Nakouti, O. Korostynska, A. Mason and A. I. Al-Shamma'a
 Built Environment and Sustainable Technologies (BEST) Research Institute
 Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK
 O.Korostynska@ljmu.ac.uk

Abstract—This paper reports on the capabilities of a novel electromagnetic wave sensing method to detect and identify the presence of various pathogenic bacteria in aqueous media. In particular, the change in the electromagnetic wave signal in microwave frequency range is used as an indicator of bacteria presence. The assessment was conducted by recording reflected signal spectra when the sensor was in contact with deionised water, *Escherichia coli*, sterile nutrient broth and *Pseudomonas aeruginosa* solutions. The distinct feature of the proposed system is that the detection is performed in real time, without the need for additional sample processing or chemicals. This bacteria detection method would be of benefit in a broad range of applications, ranging from water quality monitoring in wastewater treatment facilities to safety assurance in healthcare and food industry.

Keywords – *Pseudomonas aeruginosa*, *Escherichia coli*, real-time analysis, electromagnetic wave sensor, planar Au pattern.

I. INTRODUCTION

Escherichia coli (*E.coli*) is a gram-negative, rod-shaped bacterium that naturally colonises the intestinal tract of warm-blooded animals, including humans [1]. One million bacterial cells are present in approximately 1 g of colon and they are often excreted into the environment, where is *E.coli*'s secondary habitat [2]. It was previously believed that *E.coli* cannot survive well outside its natural environment due to hostile and antagonistic conditions, such as solar radiation, high or low temperature, poor water quality or low organic matter content [3-9]. However it is now well documented that the bacterium can not only survive but also replicate in water and on algae under different sets of temperature [10, 11].

Contamination of food and water with fecal bacteria is an ongoing problem, posing a major risk to public health. Water-borne diseases are the most common cause of mortality and morbidity worldwide. In the US 76 million of cases are reported each year, which lead to 325,000 hospitalisations and 5,000 deaths [12]. Bacterial infections that cause diarrhoea are behind 1.8 million deaths in developing countries [13]. *E.coli*

is one of the pathogenic agents causing these diseases amongst *Salmonella*, *Shigella* and *Campylobacter* [14].

In 1996 an outbreak of *E.coli* 0151:H7 in elementary schools in Osaka caused 7,900 hospitalisations and three deaths [15]. Moreover, in 2006 an outbreak of *E.coli* 0151:H7 in US and Canada was responsible for 199 infections and 3 deaths [16]. The source was spinach washed with contaminated irrigation water.

Water-borne diseases also have an economic and social impact. They lead to increased medical costs and can affect tourism through bad publicity. They also have a huge impact on agriculture. According to the US Department of Agriculture, diseases caused by the specific pathogenic agents cost the government \$6.9 billion [17].

Therefore, careful monitoring of fecal contamination in the water is crucial for both public health and economic development. *E.coli* is used as an indicator for fecal contamination and its effective monitoring is essential [18]. Current methods of *E.coli* detection are expensive and not sensitive enough. It has been reported that horizontal, fluorophore-enhanced repetitive extragenic palindromic PCR DNA fingerprinting is an accurate method for identifying the presence of *E. coli* in water, but the assay is complicated and it requires analysis in an equipped laboratory [4]. Multiplex quantitative real-time reverse transcriptase PCR for F⁺-specific RNA coliphages is another popular method, but it faces the same criticism as before [19].

Pseudomonas aeruginosa PA01 (ATCC 15692) [*P. aeruginosa*] is a gram-negative bacterium, which is motile, rod-shaped, and is recognised as an emerging opportunist pathogen of significant clinical interest [20-24]. During infection, it enters the host's bloodstream and competes for iron. *P. aeruginosa* produces siderophores, low molecular weight compounds that allow the organism to sequester the host's iron with high affinity [25]. Upon infection, *P. aeruginosa* colonises the lungs of patients suffering from Cystic Fibrosis (CF), producing an extracellular

polysaccharide that attaches to bronchial mucus, thus causing obstruction of the respiratory system. Although more infant and children with CF survive to adult life, *P. aeruginosa* is the primary cause of morbidity and mortality in such cases [26, 27]. It can also infect critical organs and the results can be fatal. *P. aeruginosa* is notoriously resistant to antibiotic treatments and it is difficult to eradicate [28-31]. Due to the nature of *P. aeruginosa*, it is important that rapid biomedical diagnostic techniques are developed in order to detect it early in its development, therefore enhancing the chance and rate of patient recovery.

Current detection of *P. aeruginosa* involves the collection of early morning sputum from the patient and analysis in a routine microbiological laboratory, with isolates form mucoid colonies on growth [32]. Although the current test is reliable, the whole procedure can be laborious and time-consuming, with up to two weeks of the expected results. DNA extraction and bacterial gene amplification methods are not yet widespread due to limitations and high cost [33]. Biomedical scientists are still looking for an alternative rapid and sensitive method [34-37]. However, current technologies that report on measurement of *P. aeruginosa* do not allow for in-situ monitoring which is vital for efficient point-of-care diagnostic provision.

In the light of the above, this paper reports on the validation of the novel electromagnetic wave sensing approach that uses bespoke planar type sensor in microwave region and is capable of determining the presence of *E.coli* and *Pseudomonas aeruginosa* in aqueous media in real time and in situ, without the need for additional sample processing or chemicals. It is based on the unique interaction of the electromagnetic field of non-thermal intensity produced by the sensor pattern with an aqueous media brought in contact with the sensor. This bacteria detection method would be of benefit in a broad range of applications, ranging from water quality monitoring in wastewater treatment facilities to safety assurance in healthcare and food industry.

II. ELECTROMAGNETIC WAVE SENSING

Electromagnetic waves propagate through low-loss dielectric materials and the amplitude of the signal reflected or transmitted through a material strongly depends on the dielectric properties of the material itself [38]. Microwave sensing has already proven itself as a valuable alternative to the current mainly laboratory based methods in a range of applications including water quality monitoring [39-43], continuous process monitoring in biogas production [44], in the food industry for the verification of the vegetable oil types [45] and in the healthcare sector for monitoring of diabetic patients' conditions [46, 47] and for non-invasive monitoring of bodily fluids [48-50].

When placed into vicinity or in direct contact with microwave sensor, a test solution interacts with the electromagnetic waves in a unique manner, which can be specifically correlated with the properties of this solution, namely with the presence of various pathogenic bacteria as in

this work. Due to this interaction, the permittivity [51] of the material changes and it manifests itself as a change in attenuated or reflected signal amplitude or a phase shift. Detailed explanation of the theoretical principles behind the electromagnetic wave sensing can be found in our recent works [42, 43, 52-54].

III. EXPERIMENTAL PROCEDURE

A. *E.coli* Preparation

Escherichia coli was inoculated into 50 ml of sterile nutrient broth (NB) and incubated for 24 h at 37 °C, 250 r/min. Following incubation, the optical density (OD) was measured at 550 nm (OD_{550}). OD_{550} of the overnight culture was 1.5.

B. *P. aeruginosa* Solution Preparation

P. aeruginosa was inoculated into 50 ml of sterile nutrient broth (NB) and incubated for 24 hours at 37 °C, 250 rpm. Following incubation, the optical density (OD) was measured at 550 nm (OD_{550}). OD_{550} of the overnight culture was 1.74. For the purposes of experimentation, this work was diluted to an OD_{550} of 1.0 to prevent possible clogging of the sensor since *P. aeruginosa* is particularly known for its ability to adhere to surfaces. For the purposes of comparison with other techniques, a 1.0 OD_{550} reading was found, via a filtration method, to be equivalent to 0.583 g/L⁻¹.

C. Microwave Sensor Head Structure

An interdigitated shaped pattern printed on FR4 substrate and operating at microwave frequencies was chosen for its versatile design that combines ease of manufacturing with desired functionality [55]. Gold was used as a metal material for both bottom layer, which acted as a ground plane, and top pattern to maintain chemical neutrality when the device is placed in contact with aqueous media with bacteria. Fig. 1 illustrates a microwave sensor on FR4 substrate with Au pattern. The sensor is connected to a cable via SMA connector. This structure also has a reservoir to contain 0.4 ml of a solution in place, where the interaction with the electromagnetic field is the strongest. A distinct feature of this sensor is its superior sensitivity to change close to the sensor surface, with this sensitivity decaying rapidly with distance away from the surface. This is advantageous as it reduces significantly the chance of undesirable factors, such as external electromagnetic signals, influencing sensor response. However, since the operation is performed at microwave frequencies, the possibilities of such interference are low.

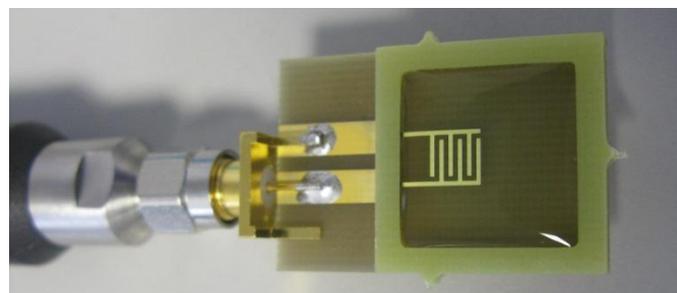


Figure 1. Microwave sensor on FR4 substrate with Au pattern, it also has a reservoir to contain the solution in place. The sensor is connected to a cable via SMA connector.

D. Measurement Setup

Rohde and Schwarz ZVA24 vector network analyser (VNA) shown in Fig. 2 was used for the purposes of data acquisition from the sensor, with this unit being appropriately calibrated according to manufacturer specifications. The data (60,000 points for each measurement) was captured in the frequency range of 0.01-15 GHz for the reflected (S_{11}) signals. A Molex edge type SMA connector [56] was used to connect the sensor via coaxial cable to the VNA. This SMA type was chosen as it is designed to excite a printed IDE sensor horizontally to maximise the available signal. The sensor and associated equipment were all specified for 50 Ω impedance. All the measurements were performed at a constant temperature of 18 $^{\circ}$ C, with all the samples being 0.4 ml in volume for consistency, as the microwave spectra depend on the volume / thickness of the test samples [57].

Each solution, namely deionised water, *Escherichia coli*, sterile nutrient broth and *Pseudomonas aeruginosa*, was measured numerous times and the results were repeatable with less than 5% deviation and reproducible. After each measurement after the aqueous sample was removed from the sensor, its response returned to the original baseline value, namely the air spectra. This suggests that there was no irreversible interaction between the solutions and the sensor itself and therefore it can be reliably reused. Notably, average sensor responses are depicted in the graph shown in the following section.

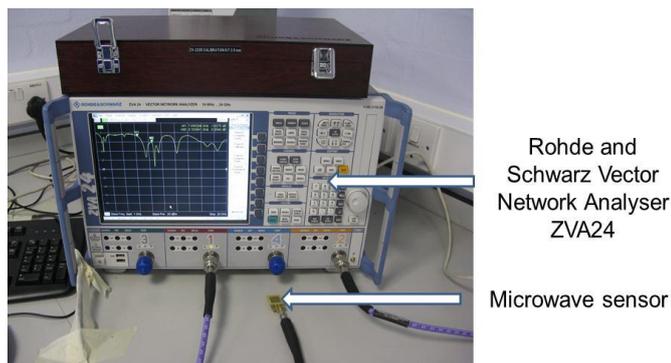


Figure 2. Measurement setup showing ZVA24 connected to a microwave sensor via coaxial cable.

IV. RESULTS AND DISCUSSION

Fig. 3 illustrates S_{11} signals distribution recorded from the bespoke microwave sensor in 0.01-15 GHz frequency range when in contact with deionised water, *E.coli*, sterile nutrient broth and *P. aeruginosa* solutions. All four spectra, each representing the average of multiple measurements, are plotted on common graph to illustrate that the proposed electromagnetic wave sensing system has the potential to differentiate between various pathogenic bacteria. As one can see, each sample has a unique response to the microwave signal resulting in resonant peaks occurring at different frequencies and having different signal amplitudes. Thus, the first major resonant peak for deionised water was recorded at frequency of 5.56 GHz, whereas it was 4.39 GHz for *E.coli*,

2.81 GHz for nutrient broth and 2.22 GHz for *P. aeruginosa*. At higher frequencies, in the region of 8.5-10 GHz, the biggest resonant peaks were recorded for all the solutions. These are: 9.63 GHz for deionised water, 9.82 GHz for *E.coli*, 8.21 GHz for nutrient broth and 8.44 GHz for *P. aeruginosa*.

Having maintained all other experimental parameters constant, the only explanation to these shifts is that they are connected with the properties of the solution under the test, namely its composition or bacteria. This particular feature makes the developed sensor an attractive option for real-time monitoring of bacteria presence in the aqueous media.

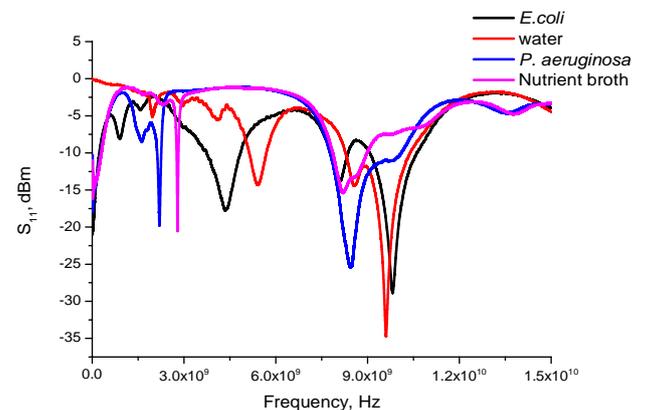


Figure 3. S_{11} signal distribution of microwave sensor in 0.01-15 GHz frequency range when in contact with deionised water, *Escherichia coli*, sterile nutrient broth and *Pseudomonas aeruginosa* solutions.

The system could potentially be made portable for a range of real-time bacteria presence monitoring purposes, when combined with hardware to replace the VNA used in this work, as demonstrated by the authors previously [58].

Notably, once the presence and the type of bacteria are recorded by the proposed sensing system, a complimentary microfluidic based system can be put in place of concern for continuous real-time monitoring of the bacteria concentration, to reveal the progress of wastewater treatment, for example, or to ensure that there is no further contamination with the pathogenic bacteria.

Such as system was recently reported by the authors in [59]. It is based on the same electromagnetic wave sensing principle, but the sensor structure used was different to allow the test fluid to pass through the sensor in an automated microfluidic channel. The sensor was embedded in a bespoke fluidic cell constructed of polymethyl-methacrylate (PMMA), which has high-performance liquid chromatography (HPLC) compatible inlet and outlet ports in order to allow fluid to pass through the cell and come into contact with the sensor device itself, as illustrated in Fig. 4 [59]. The system was able of instantaneous diagnostics of *P. aeruginosa* concentration in the range of $OD_{550} 25 \times 10^{-3} - 1.0$. Results from the data collected at this sensor's resonant peak of approx. 292 MHz are shown in Fig. 5 [59]. Results from all the measurements repetitions were averaged and error bars show the range of readings at each concentration.

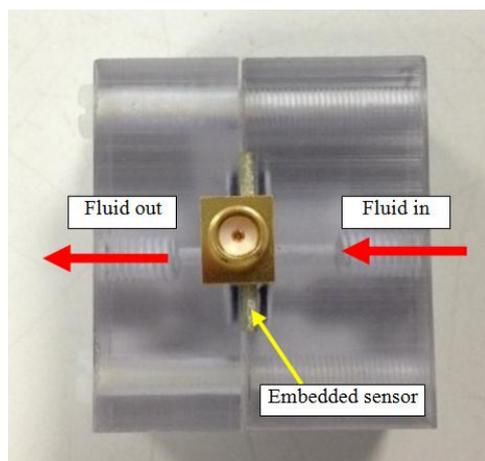


Figure 4. The sensor and fluidic cell combination [59].

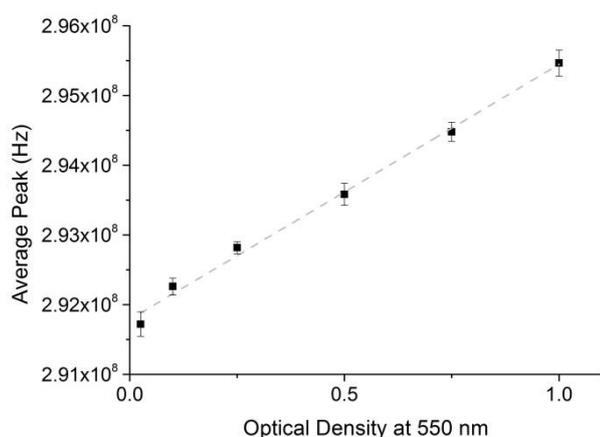


Figure 5. Plot of average resonant peak with changing *P. aeruginosa* optical density. Error bars indicate the range over which measurements varying during repetitions [59].

Notably, depending on the desired accuracy of the microwave signal, number of points per spectra can be varied. Even when maximum 60,000 points per spectra are being recorded, the process takes 2-3 seconds per whole frequency sweep, with 10 sweeps recorded and averaged being the common approach to eliminate any possible random signals. This fast response makes the proposed system virtually real-time. Based on the results presented above, one can firmly suggest that the developed electromagnetic wave sensing system is capable of instantaneous monitoring of bacteria presence in aqueous media and is a viable alternative to currently used laboratory based methods of bacteria quantification and qualification. Further research is underway to assess the capabilities of the system to identify other types of bacteria.

CONCLUSION

This paper reports on a bespoke electromagnetic wave sensing method that is capable to detect and identify the presence of various pathogenic bacteria in aqueous media. The system was tested on deionised water, *Escherichia coli*, sterile nutrient broth and *Pseudomonas aeruginosa* solutions. The distinct feature of the proposed system is that the detection is

performed in real time, without the need for additional sample processing or chemicals. This bacteria detection method would be of benefit in a broad range of applications, ranging from water quality monitoring in wastewater treatment facilities to safety assurance in healthcare and food industries.

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