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A Waveguide Resonator Sensor for Bacterial Growth Monitoring: Towards Antibiotic Susceptibility Testing

O. Niksan¹, J. Fowler¹, V. Balasubramanian¹,
A. Shah¹, S. Pakpour¹, and M. Zarifi^{1*}

¹University of British Columbia, Canada

Outline

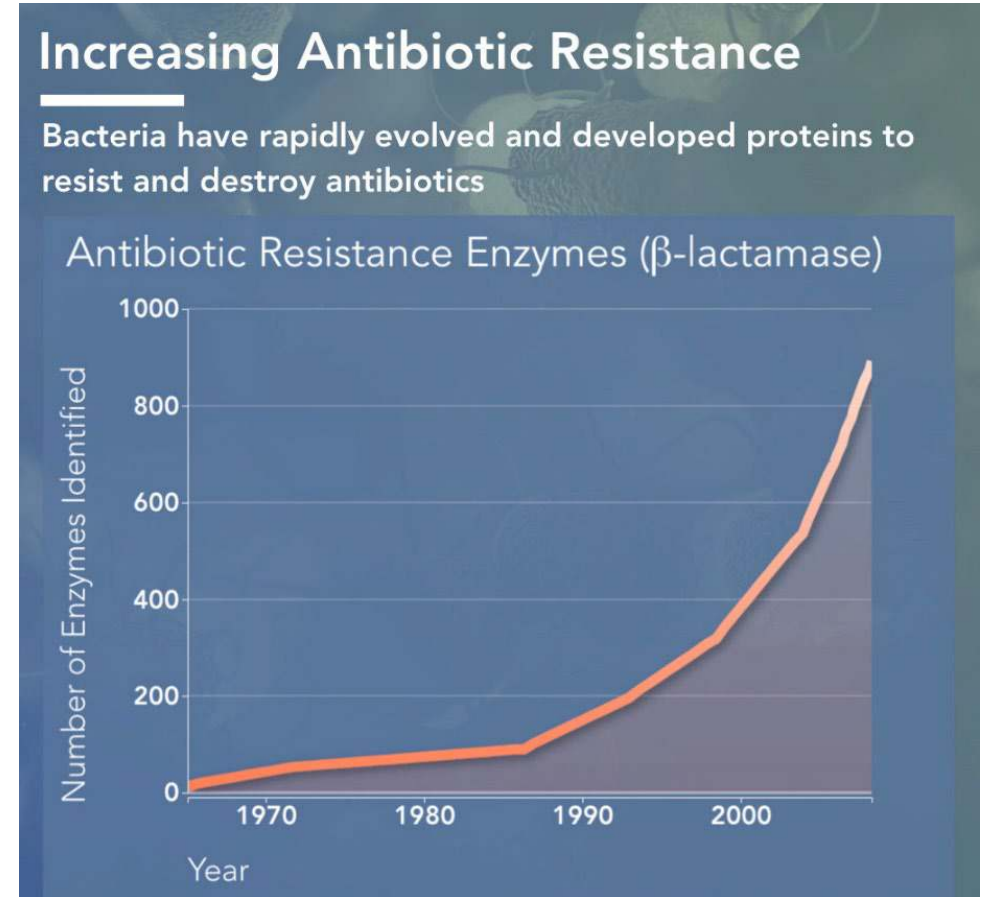
- Field of Challenge in Antibiotics Resistance
- Resonance-based microwave sensors
- Planar sensors for bacterial growth
- Proposed Waveguide/Resonator Sensor
- Results and Observations
- Outcomes

Bacteria species are **fighting back!**

Efficacy of antibiotics has started to decrease.

This is due to:

- Antibiotic overuse (30% to 50% prescriptions are unnecessary)
- Inappropriate prescriptions
- Stalled development of new antibiotics
- Estimated number of illnesses and deaths:
 - **2,049,449** and **23,000**



The Science of Antibiotic Resistance. Bacteria can develop proteins and enzymes that protect themselves and destroy antibiotics [1].

[1]: CDC.gov

A primary knowledge gap in the fight against ABR is [1]:

ADVANCED TECHNOLOGIES that can identify threats **much faster** than **current practices**.

Drawbacks of current bacterial growth monitoring (BGM) methods [2]:

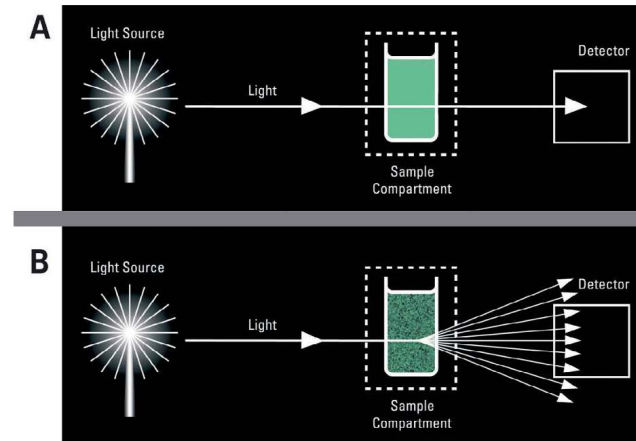
- time-consuming,
- low throughput,
- contact-based [2].

Similarly, present antibiotic susceptibility testing (AST) methods impose [3]

- false positives,
- slow response
- high cost



A disk diffusion (AST) test for E. coli. The diameters include information on the susceptible, intermediate, or resistant behavior.



BGM using Light scattering spectrophotometry.

[1]: CDC.gov

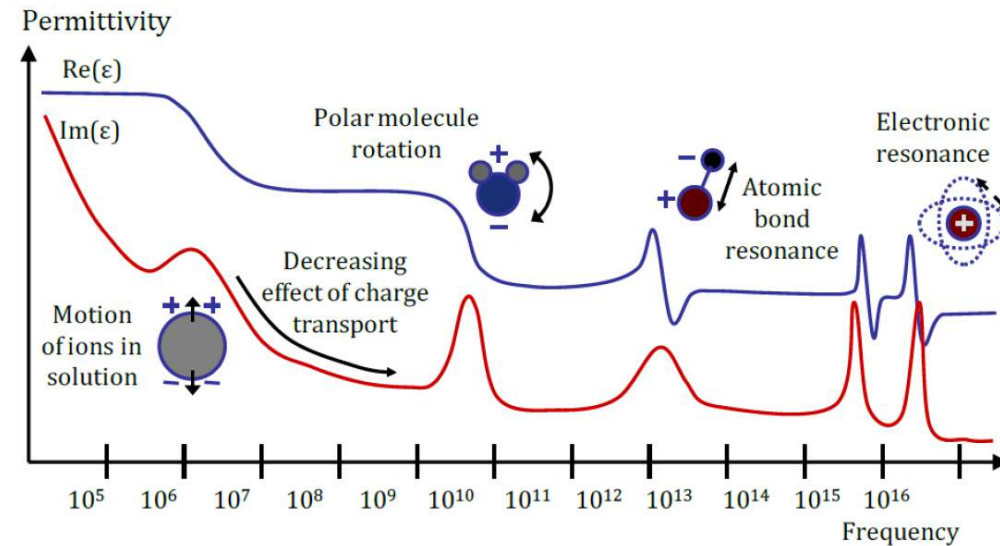
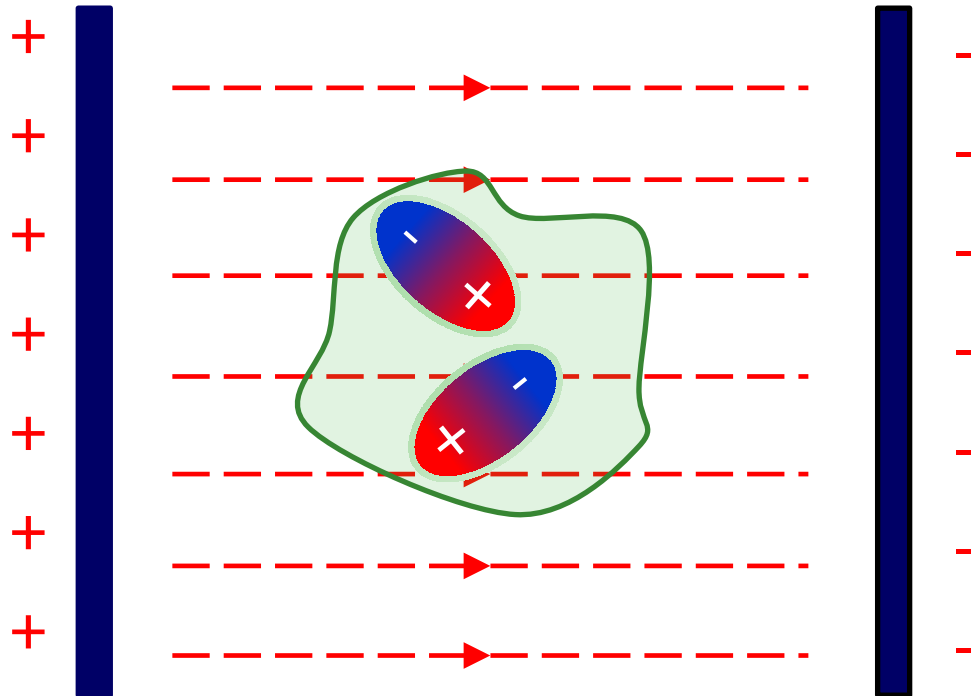
[2]: Narang et al. (10.1038/s41598-018-34001-w)

[3]: Reller et al. (10.1086/647952)

- The interaction of materials with alternating electromagnetic fields in the microwave regime can be characterized by (complex) permittivity.
- The measured permittivity values can be used to differentiate materials.

$$\varepsilon_r = \varepsilon' - j\varepsilon''$$

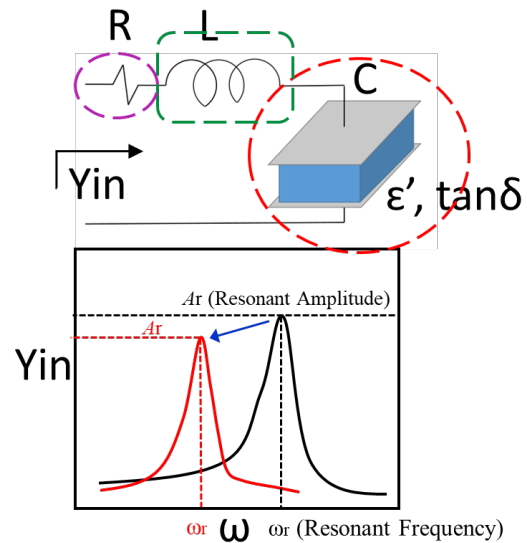
$$\tan \delta = \frac{\omega\varepsilon'' + \sigma_{DC}}{\omega\varepsilon'}$$



Planar and cavity resonator sensors

- A resonant structure can be perturbed by materials in the proximity.
- The perturbation can be characterized by probing the resonator and monitoring S-parameters.

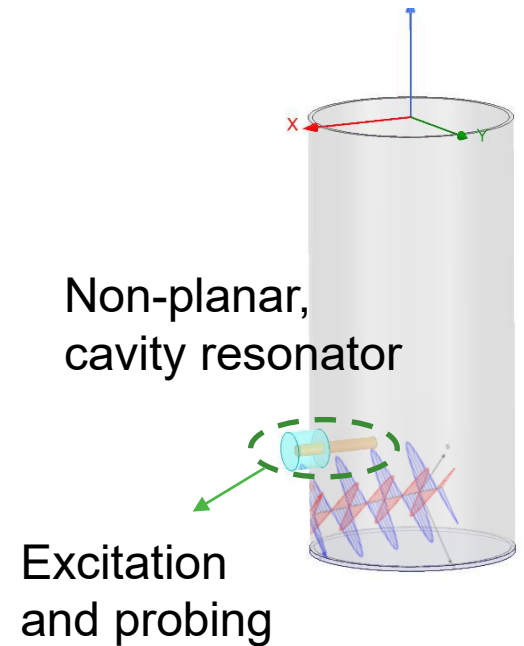
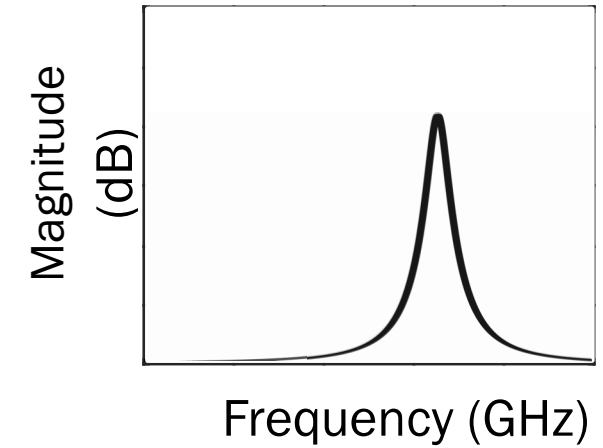
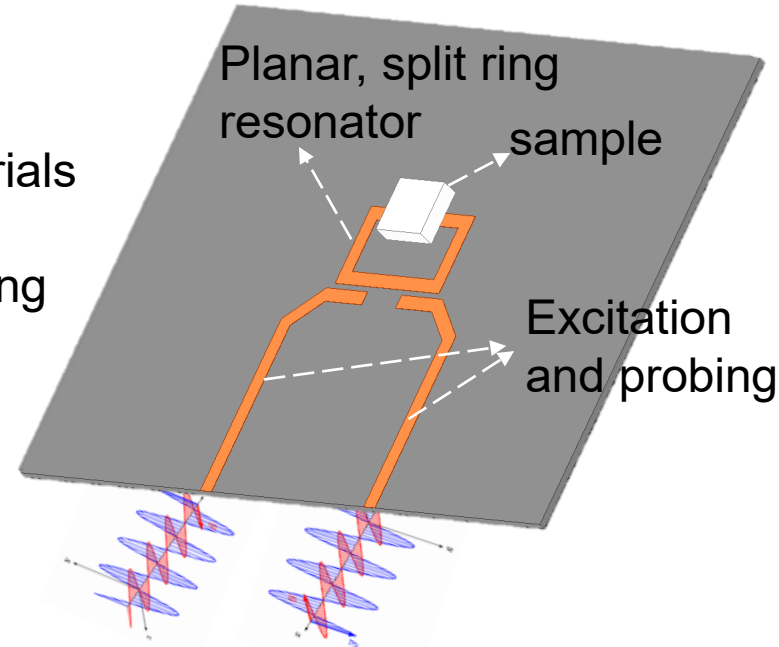
The resonant and sensing behavior can be described by a lumped RLC model:

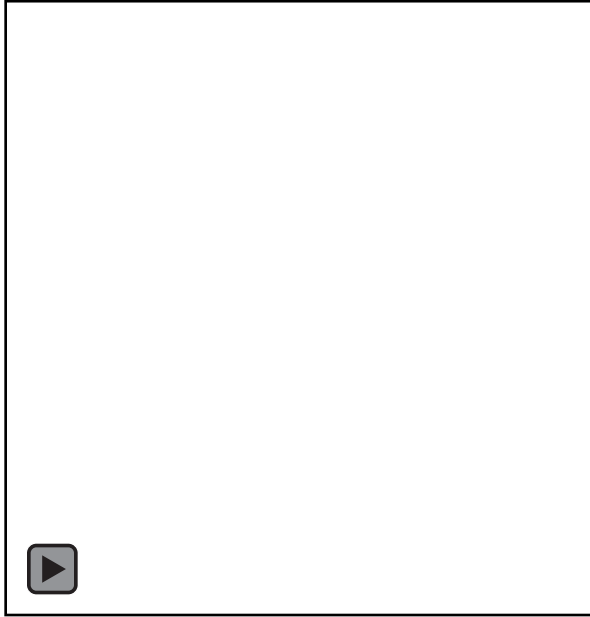


$$\omega_r = \sqrt{\frac{\epsilon'}{L(\epsilon'^2 + \epsilon''^2) \frac{A}{d}}} = \frac{1}{\sqrt{L \epsilon' \frac{A}{d} (1 + (\tan \delta)^2)}}$$

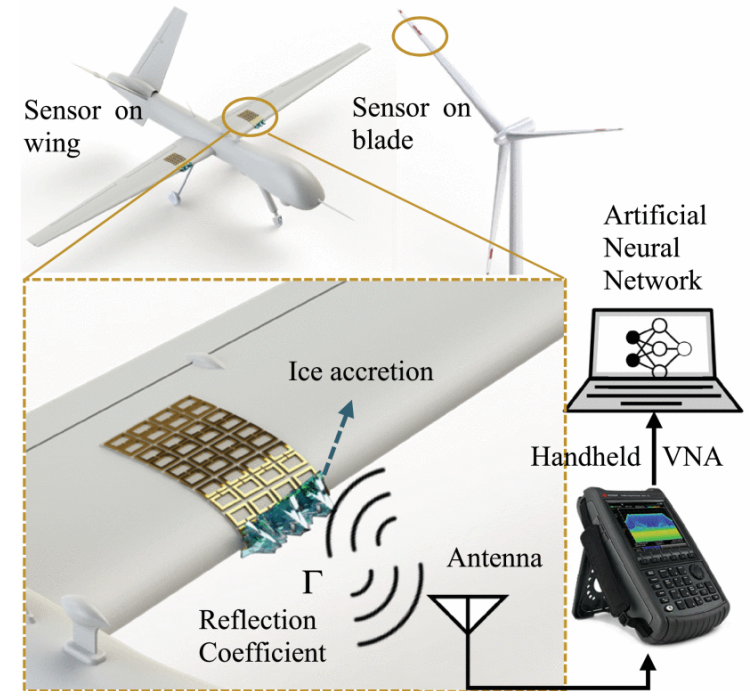
$$A_s(\omega_r) = R + \frac{\epsilon''}{\sqrt{\frac{\epsilon'}{L} (\epsilon'^2 + \epsilon''^2) \frac{A}{d}}} = R + L \cdot \omega_r \cdot \tan \delta$$

$$\text{where } \tan \delta = \frac{\epsilon''}{\epsilon'} + \frac{\sigma/\omega}{\epsilon'} \approx \frac{\epsilon''}{\epsilon'} \text{ for } \frac{\sigma/\omega}{\epsilon'} \ll \frac{\epsilon''}{\epsilon'}$$



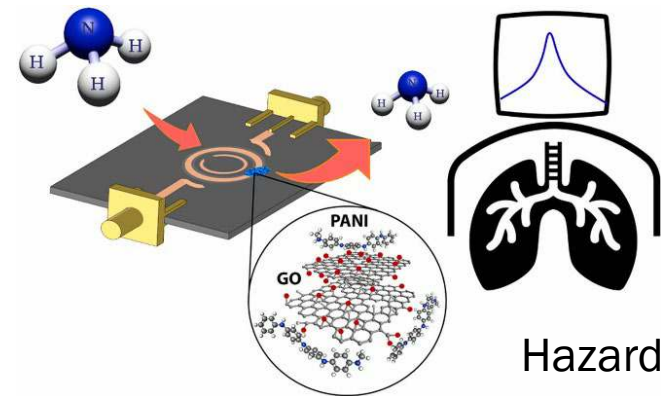


- Non-contact sensing
- Real-time operation
- Improved dynamic range (spectroscopy)



Wireless, battery-free, AI-integrated Ice sensor [5].

Wireless, Kirigami-enabled strain sensor [4].



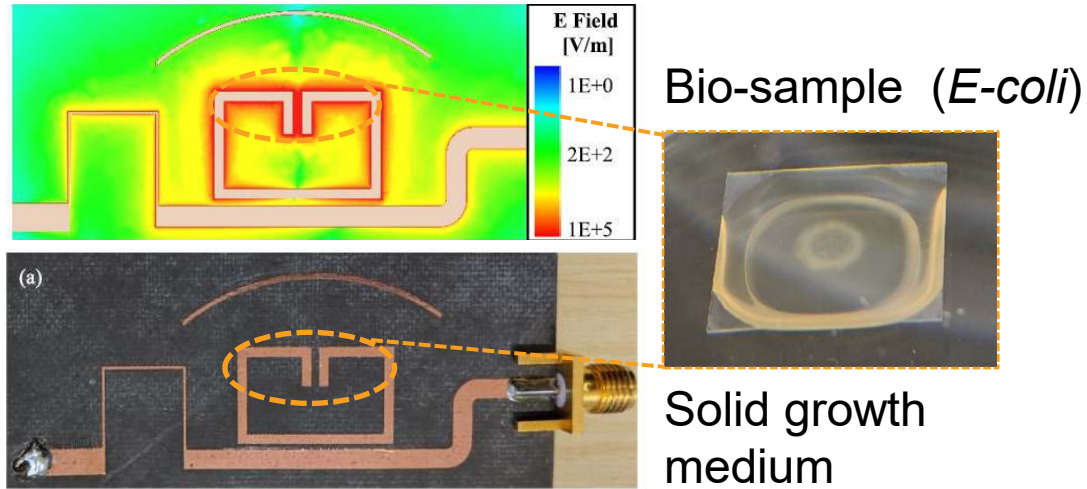
Hazardous Vapors and Gas sensor [6].

[4]: Dijvejin et al ([10.1021/acsami.0c10384](https://doi.org/10.1021/acsami.0c10384))

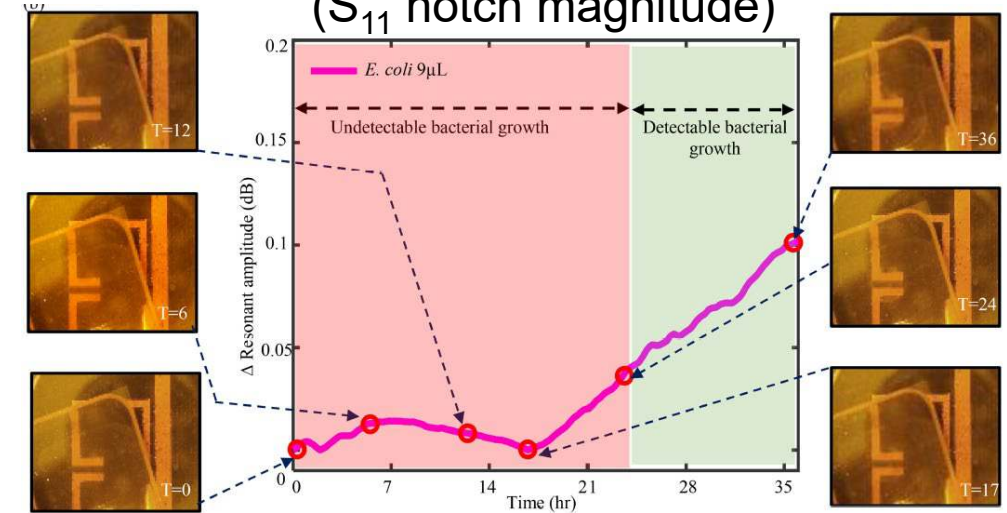
[5]: Niksan et. al ([10.1109/TMTT.2022.3222194](https://doi.org/10.1109/TMTT.2022.3222194))

[6]: Javadian et. Al ([10.1016/j.jhazmat.2021.126283](https://doi.org/10.1016/j.jhazmat.2021.126283))

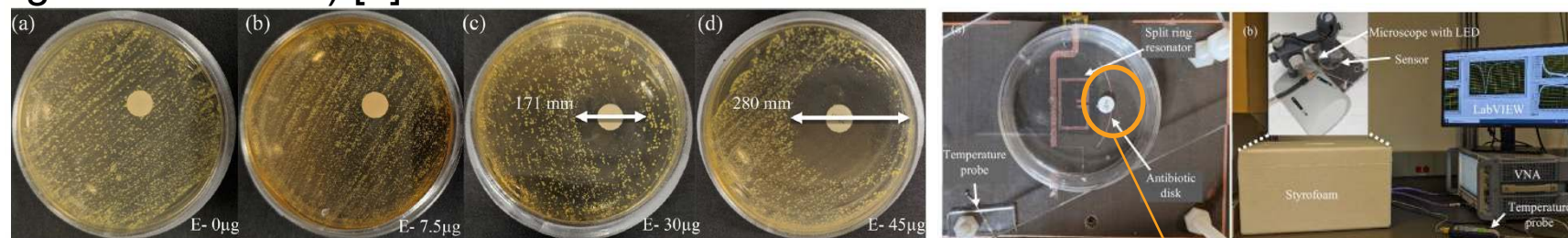
Planar split ring resonator (SRR) sensor [7]:



Detection of growth from resonance parameters (S_{11} notch magnitude)



Detection of growth inhibition, in proximity to antibiotics (solid growth medium) [8]:



Limitations:

- Solid growth medium
- Limited sample geometries
- Prone to noise and interference

Images of inhibited *E-coli* growth near antibiotics

Antibiotic disk

[7]: Jain et al. (10.1109/TBCAS.2021.3055227)

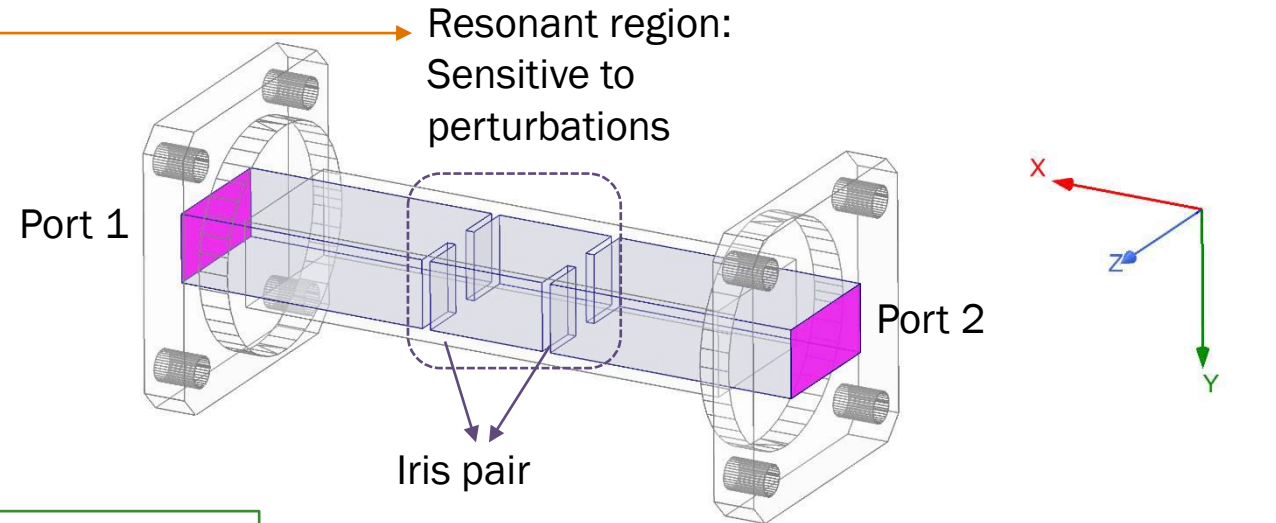
[8]: Jain et al. (10.1038/s41598-021-94139-y)

Waveguide resonator sensors

- The medium between two iris pairs can be a resonant section.

The key **advantages** of this design:

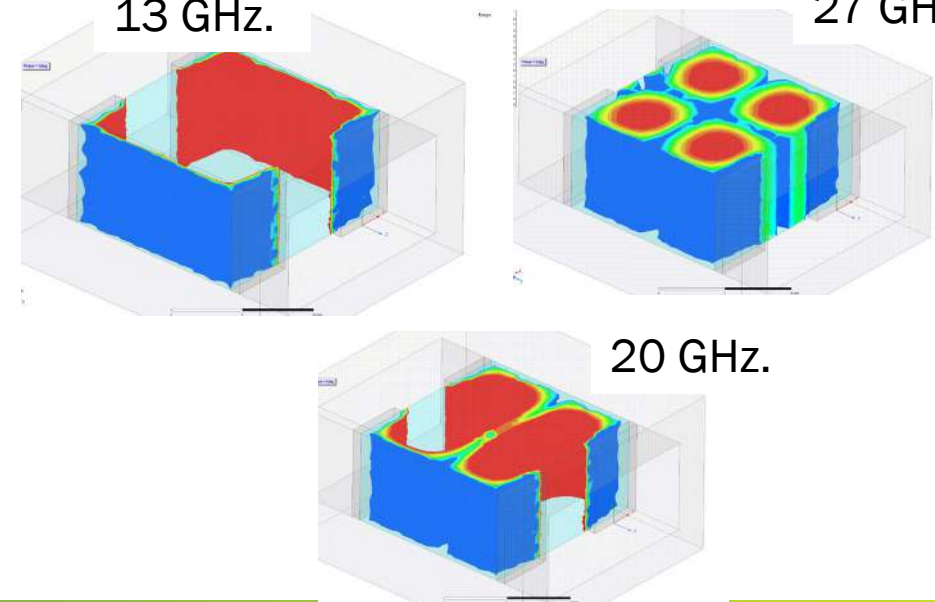
- Mechanical reconfigurability (dimensions and iris distance)
- Resonant mode selection for non-homogenous material samples
- Shielded (noise and interference)



13 GHz.

27 GHz.

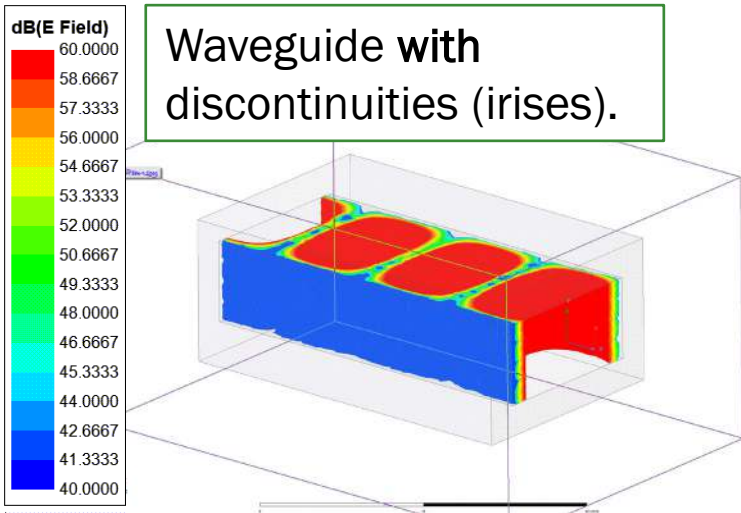
20 GHz.



Waveguide **without** discontinuities (irises).

Resonant section (i.e., standing wave) is generated.

Waveguide **with** discontinuities (irises).



Proposed Waveguide resonator sensor

The resonant frequency:

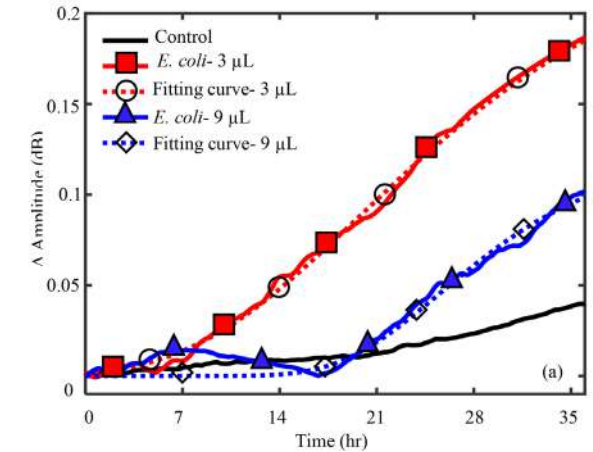
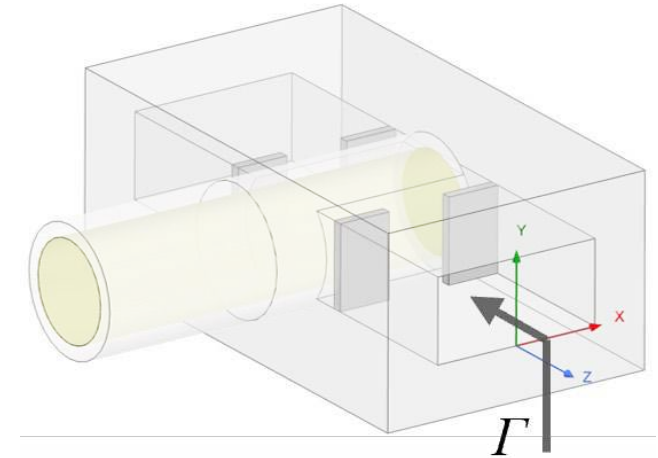
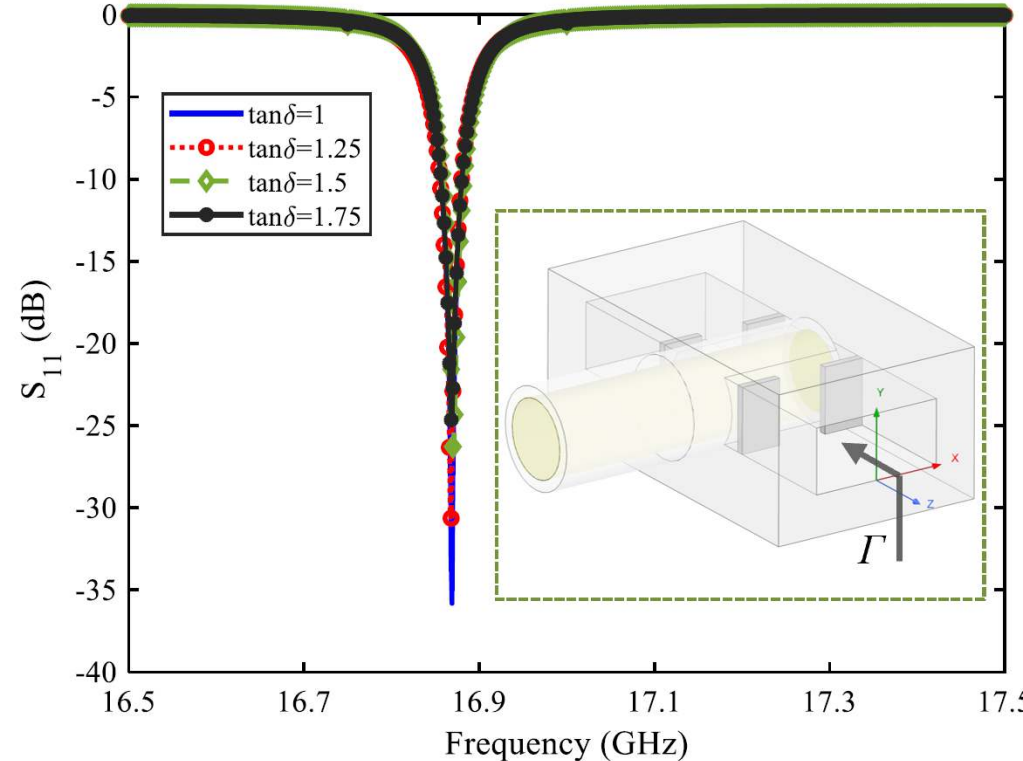
$$f_{mnp} = \frac{c}{2\pi\sqrt{\epsilon_r\mu_r}} \sqrt{\left(\frac{m\pi}{a}\right)^2 + \left(\frac{n\pi}{b}\right)^2 + \left(\frac{p\pi}{d}\right)^2}$$

The sensitivity can be described by cavity perturbation theory (material perturbations):

$$Q = \frac{4\pi f W_e}{P_d} = \frac{\epsilon'}{\epsilon''} = \frac{1}{\tan \delta}$$

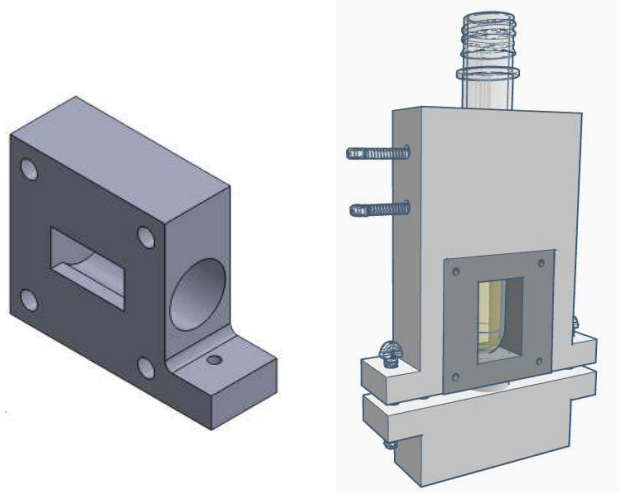
The resonant characteristics affect the reflection coefficient of the waveguide:

$$\Gamma = \frac{Z_{\text{resonator}} - Z_0}{Z_{\text{resonator}} + Z_0}$$

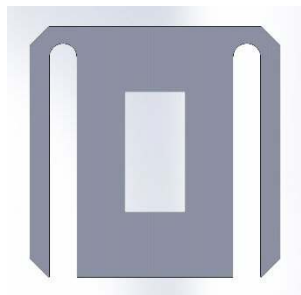
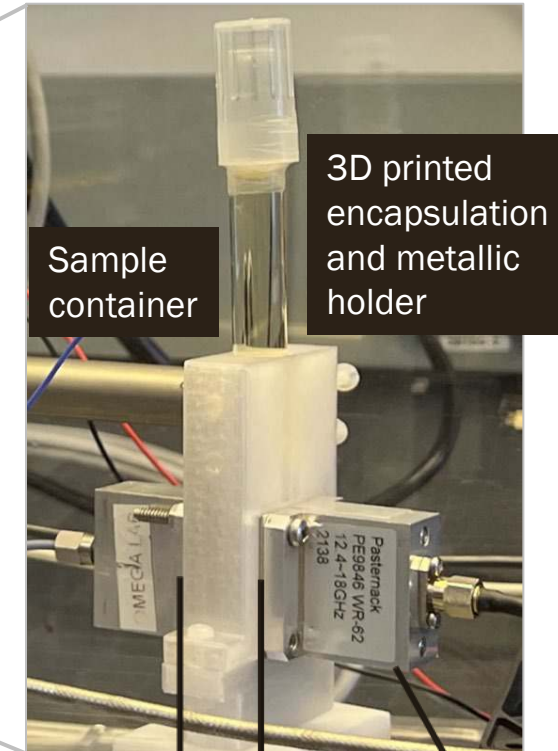
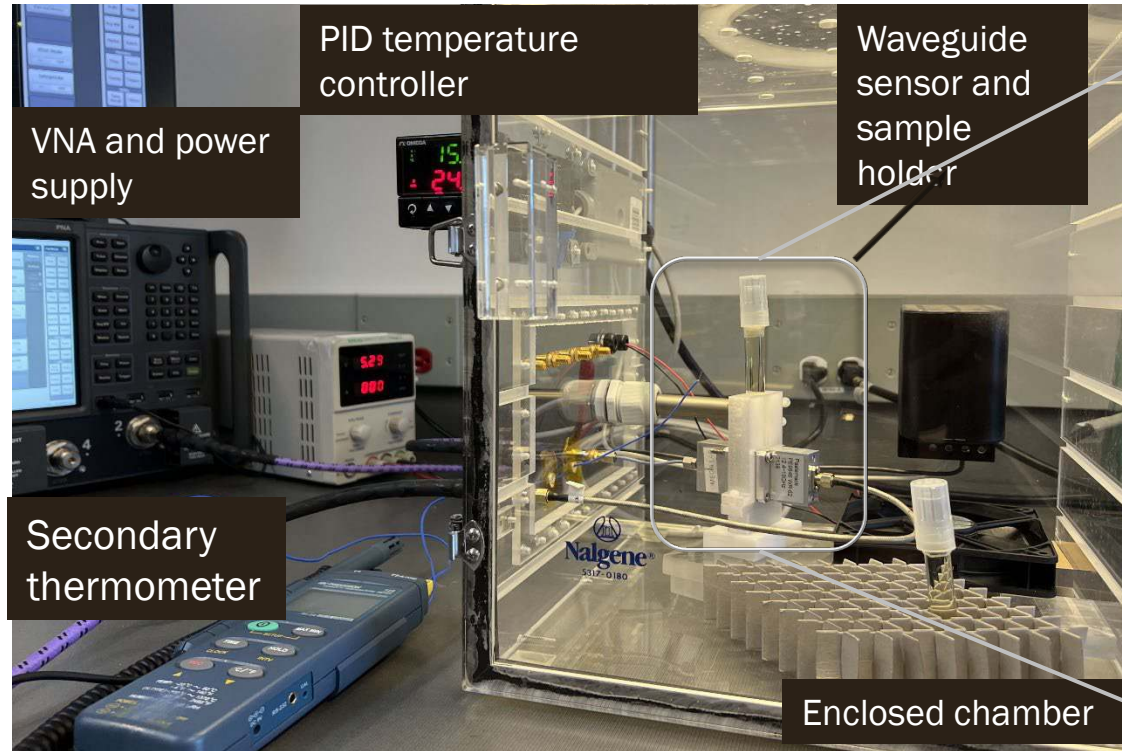


Bacterial growth, modeled as variations in the loss tangent [7].

Experimental Investigation



Metallic waveguide (offset) section, encapsulated by 3D printed holder.



Designed and machined irises for facile replacement and response tuning (impedance matching)



Side view of the sample holder (+metallic offset section)

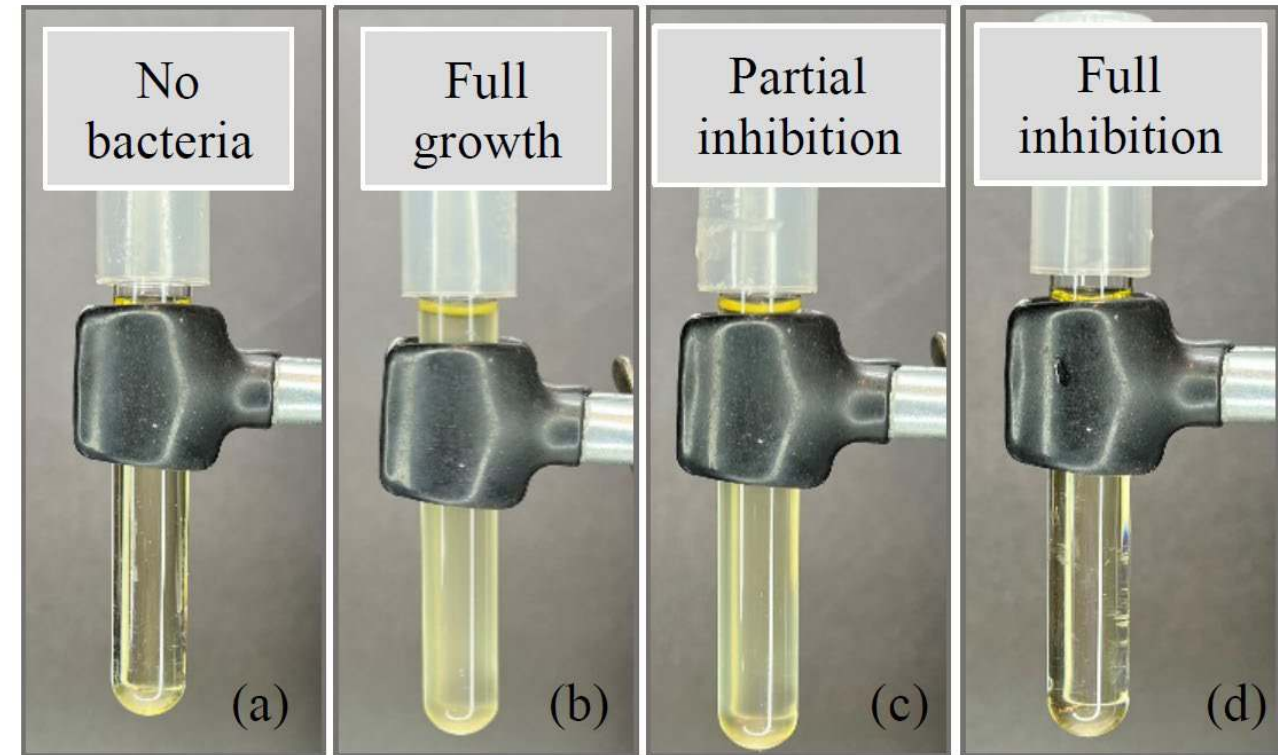
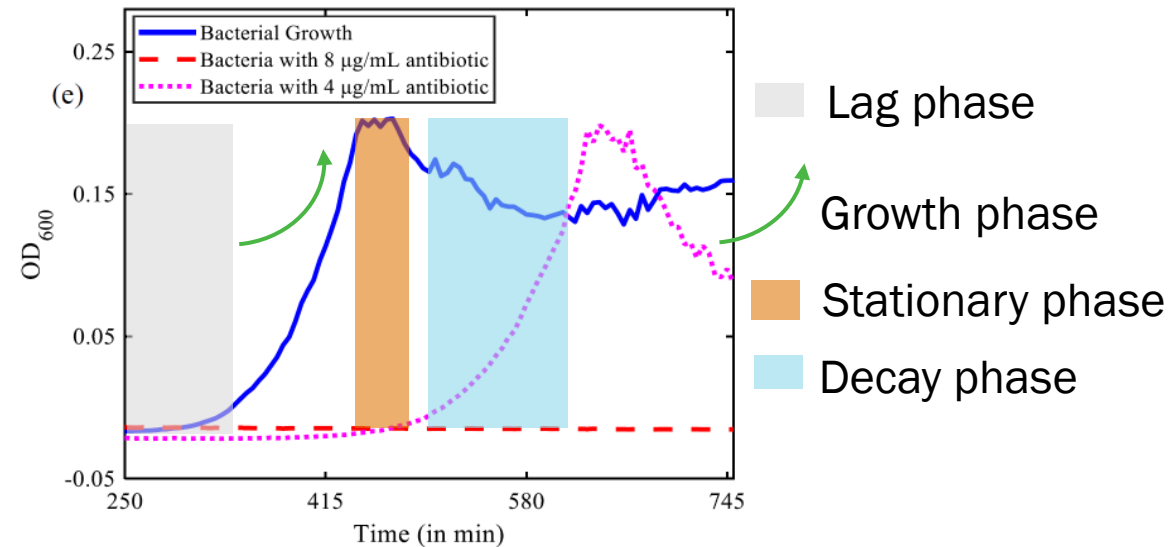
Experimental Investigation

- 12-hour OD measurements using the Agilent Cary 3500 UV-Vis Spectrophotometer, at 37 °C
- 4 units/ml penicillin and 4 $\mu\text{g}/\text{mL}$ streptomycin:

Partial inhibition

- 8 units/ml penicillin and 8 $\mu\text{g}/\text{mL}$ streptomycin:

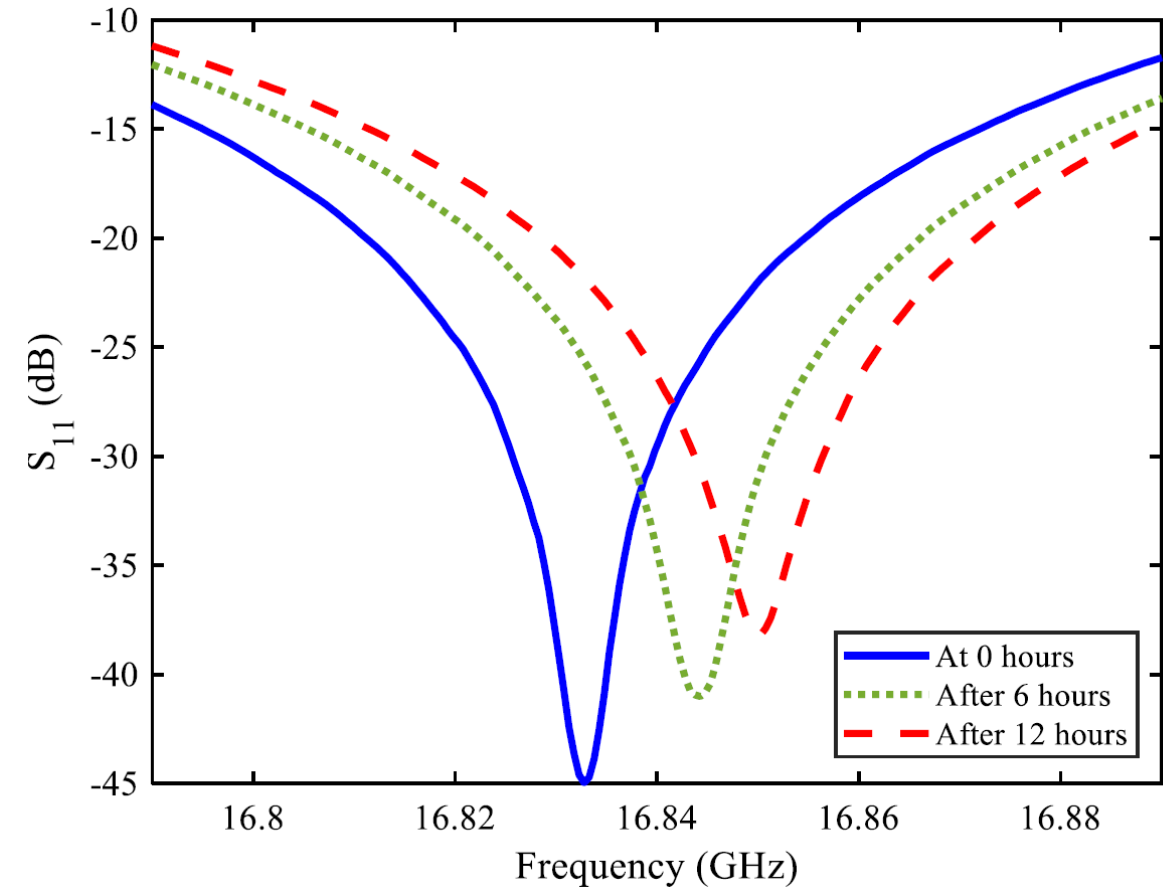
Full inhibition



Photographs of the test samples for (a) no growth, (b) full growth without any antibiotics, (c) partial inhibition by antibiotics, and (d) full inhibition by antibiotics.

Steady bacterial growth with no inhibition:

- 50 μL of stock culture (*E. coli* ATCC® 11229™) was inoculated in 5 mL of Luria-Bertani (LB) broth.
- The bacterial culture was allowed to grow in the LB media at 37 °C.
- This data is absolute, i.e., not adjusted for the LB baseline and the effect of temperature variations in the chamber.



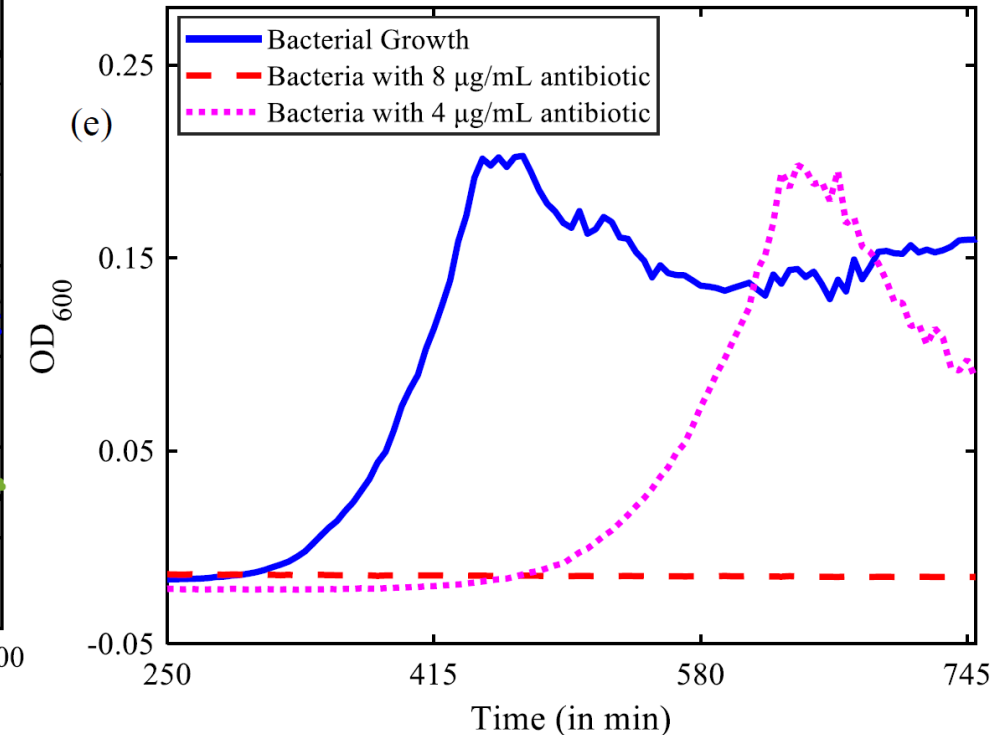
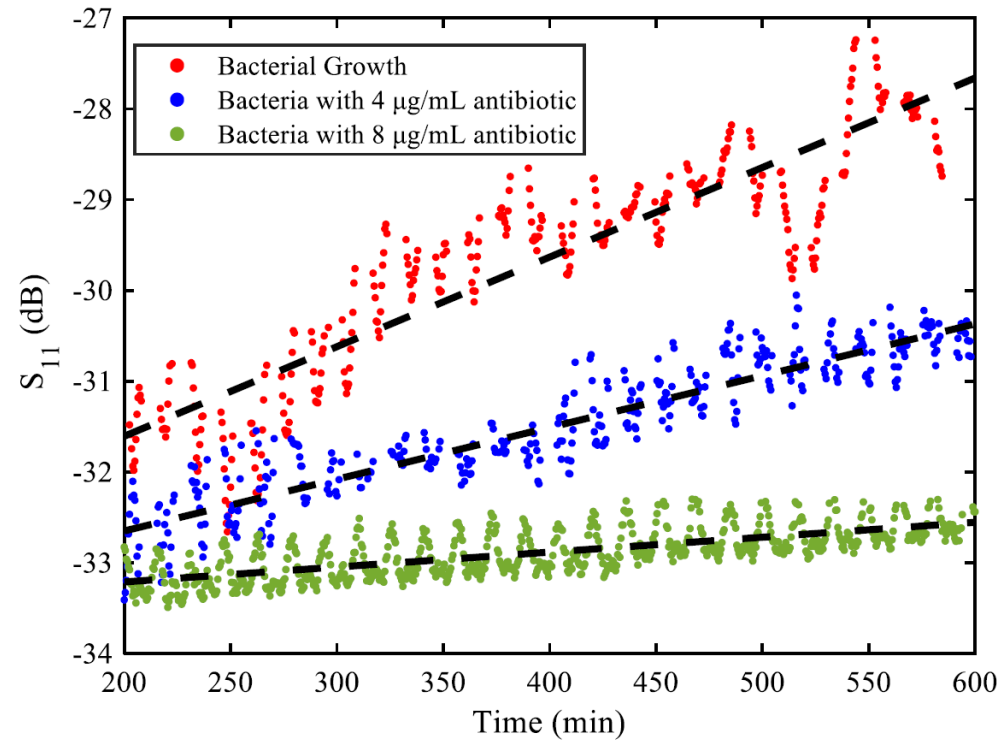
Measured S_{11} (dB) after, 0, 6, and 12 hours of placing bacteria-containing test tubes in the resonator.

The growth experiments were performed with test samples and two different volumes of antibiotics.

The effects of lag phase in bacterial growth, temperature fluctuations, and mechanical stability were nullified in the post-processing.

Three different trends were observed:

- **full growth** (0 $\mu\text{g/mL}$ antibiotics)
- **partial-inhibition** (4 $\mu\text{g/mL}$ antibiotics)
- **full inhibition** (8 $\mu\text{g/mL}$ antibiotics).



- The iris-based waveguide sensor detected growth without extracting or exposing the bio-hazard material to reacting agents (non-contact sensing).
- The variations of the resonant characteristics can be correlated with the growth factor of microbiomes.
- The observed correlations potentiate the development of reliable microwave-based Antibiotic susceptibility testing method.
- Based on core microwave interaction with bio-samples, sample properties (e.g., transparency) are not a limiting factor.
- A robust structure, shielded from environmental noise and interference.



Omid Niksan



James
Fowler



Vishal
Balasubramanian



Prof. Sepideh Pakpour



*Prof. Mohammad H.
Zarifi*