Rapid Detection of Bacterial Response to Antibiotics through Induced Phase Noise of a Resonant Crystal

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***Abstract*—A new low-cost, multi-channel desktop digital instrument was developed that measures the response of bacteria to multiple antibiotics in about an hour by detecting phase noise induced by mechanical motion of the bacteria when tightly tethered to a resonator. The motivation for this work comes from the approximately 13 million urinary tract infections (UTIs) leading to some 63,000 deaths yearly in the USA and many more worldwide, primarily due to *E. Coli* and the two-day delay in diagnosing antibiotic resistance. Rapid determination of efficacy could potentially save lives and extend antibiotic lifetimes.**

Keywords—Urinary tract infections, antibiotic resistance, *E. Coli.*, crystal resonator, phase noise, antimicrobial susceptibility testing

I. INTRODUCTION

In this paper, a very sensitive technique is described for rapid detection of the response of bacteria to antibiotics when closely tethered in solution to a crystal quartz resonator. The first data were obtained with a relatively large component-based laboratory system using 5 MHz quartz resonators, operating in the fundamental thickness-shear mode, and samples of cultured *Escherichia. Coli (E. Coli)* [1].

Based on success of our laboratory experiments at the National Institute of Standards and Technology (NIST) on *E. Coli*, a new, small, low-cost, desktop digital instrument was developed at Microbial Pulse Diagnostics (MPD) that incorporates many new automated features and could be manufactured. First results with this improved and expanded system show the same form of time-dependent response of *E.coli* to ampicillin as seen previously with the component system and commercial cassettes.

The motivation for this work is a need for more rapid clinical assessment of the efficacy of antibiotics for treating infections. Each year in the USA alone, approximately 13 million urinary tract infections (UTIs) are reported, and 63,000 of these infections lead to death [2]. The majority of UTI infections are caused by *E. Coli.* Untreated UTIs progress in some patients to sepsis where the mortality rate increases about 8%/hour without an effective antibiotic [3]. Rapid diagnosis is critical, but the standard technique used all over the world takes 2 days, forcing health care professionals to guess the appropriate antibiotic. UTI pathogens display resistance rates to front line UTI antibiotics ranging from 10 to 70% [4-9], leading to many instances where an initial prescription is ineffective. Patients face increasingly complicated infections, extended hospitalizations and, sometimes, death. This problem is becoming even more serious as an increasing number of antibiotic-resistant strains of bacteria have been observed, leading to more serious infections, longer hospital stays, and higher death rates. Moreover, fewer new antibiotics are being developed than in past years. The direct health care costs exceeded 3.5 billion in 2019 for the USA [10].

If rapid determination of effective antibiotics could be made, potentially many lives could be saved and the effective lifetime of our present arsenal of antibiotics extended, all with a significant saving in health care costs.

II. MEASUREMENT CONCEPT

The idea of using a quartz crystal resonator to probe the vitality of *E. Coli* bacteria originated with Ward Johnson at NIST. The mechanical motion of live bacteria adhered to the surface of a high Q resonator causes small, time dependent perturbations to the

surface stress, which lead to small changes in the instantaneous frequency of the resonator. In the presence of an effective antibiotic, the added PM noise is greatly reduced.

If a resonator is excited by a noiseless source, an induced phase change is observed across the resonator, which near resonance is given by

$$\Delta\varphi(t,\omega_0) \approx 2Q\left[\frac{\omega_0-\omega_{R(t)}}{\omega_0}\right] \quad (1)$$

where $\omega_R(t)$ is the resonant frequency of the resonator, ω_0 is the frequency of the driving source, and Q is the loaded Q factor of resonator.

The small time-dependent bacteria-induced phase shift can be separated from the excitation signal using a phase bridge with the resonator in one leg, as schematically shown in Fig. 1. The bridge is driven by a low-noise tunable frequency source operating near the resonant frequency of the resonator. In addition to the direct signal through the resonator, there is a parallel component from the capacitance of the electrodes and mounting. This competing contribution is cancelled using a 180-degree power splitter and an adjustable capacitor. The through signal and the cancellation signal are combined at the input of a low-noise RF amplifier.

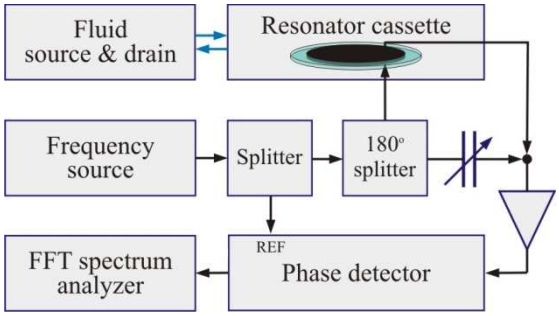


Fig 1. Schematic of PM noise measurement system.

The phase detector compares the phase of the input excitation signal with that of the resonator signal. The output of the phase detector is then processed in a Fast Fourier Transform (FFT) spectrum analyzer, yielding the power spectral density of Phase Modulation (PM) noise. For calibration purposes, the tunable source is Frequency Modulated (FM) at a frequency between 250 and 340 Hz, depending on the apparatus.

The power spectral density of the signal is

$$2\,L(f) = S_\varphi(f) = 4\,Q^2\left[S_{y\omega_R}(f) + S_{y\omega_o}(f) + S_{yCal}(f)\right], \quad (2)$$

where $L(f)$ is the single-sided power spectral density of PM noise, $S_\varphi(f)$ is total power spectral density of PM noise, and $S_{y\omega_R}(f)$, $S_{y\omega_o}(f)$, and $S_{yCal}(f)$ are, respectively, the power spectral density of fractional frequency noise from the resonator, the source in the absence of the FM calibration tone, and the calibration tone.

Calibration of the measurement via the FM calibration tone enables multiple sensors to be operated in parallel with portions of the same bacterial sample but different antibiotics. The time-dependent response from the multiple sensors can then be rapidly and accurately compared to determine which antibiotics are most effective in treating a specific bacterial infection.

The measurement cycle is given below:

1. Tune the source to line center for each resonator
2. Calibrate each measurement using FM on source
3. Determine baseline PM noise in phosphate-buffered saline (PBS) without bacteria
4. Load bacterial samples on test and control resonators
5. Measure and record live bacteria PM noise
6. Introduce different antibiotics in all but control sensor
7. Continue recording bacteria PM noise data
8. Compute PM integral (minus initial baseline from 3) over selected frequency range and plot

Fig. 2 shows typical PM power spectra for a measurement cycle for a Test resonator exposed to polymyxin B (PMB) in Lysogeny broth (LB) and a Control resonator in LB. To compare changes in microbe-related signals over time, the baseline PM noise in PBS is first subtracted from each spectrum. The spectra are then converted to $S_\varphi(f)$ and integrated from 4 Hz to 20 Hz. This integrated spectral power is plotted in Fig 3.

Polymyxin B kills *E. Coli* cells by punching holes in the cellular wall but leaves the carcass relatively intact. The cellular coverage on the resonator therefore stays about the same while the added PM noise drops nearly to zero as shown in Fig. 3 [1].

Fig. 3 shows that the detected signal is due to the internal mechanical motion of the cells and not Brownian motion because the added PM noise drops to near zero while cellular coverage is the same after introduction of polymyxin B [1].

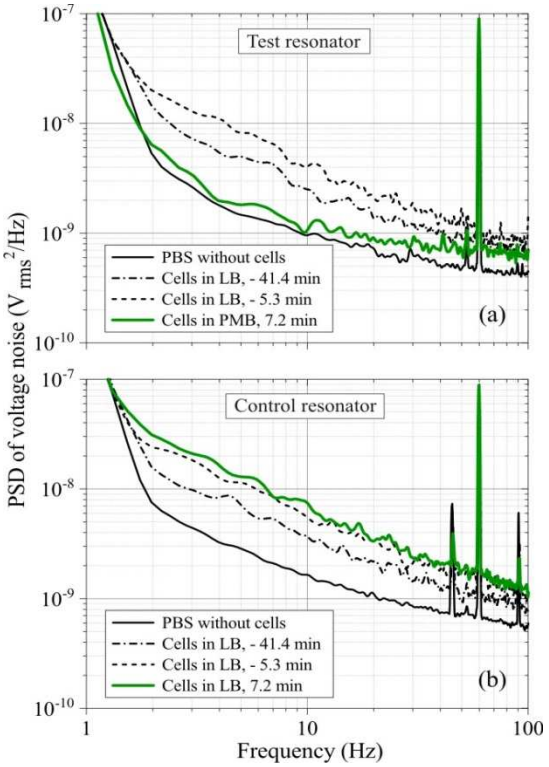


Fig. 2. Power spectral density PSD of voltage noise for antibiotic PMB on *E. Coli* for test and control resonator

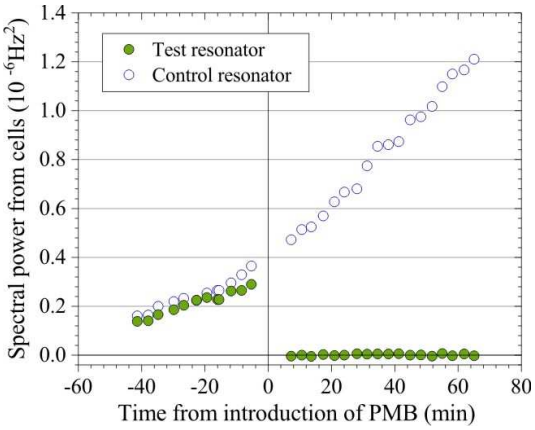


Fig. 3 Integrated $S_\varphi(f)$ 4-20 Hz for PMB treatment of *E. Coli*.

The results of Figs. 2-3 were obtained at NIST using a large rack-mounted analog system attached to the phase bridge mounted on an optical table. However, such a system isn't suitable for making multiple channel measurements necessary to simultaneously test multiple antibiotics against an infection, so, a new approach was needed.

III. NEW DESKTOP INSTRUMENT

MPD embarked on developing a new desktop instrument using digital techniques. The first implementation of the new instrument shown on the right in Fig. 4 presently has 4-8 channels but could be expanded to accept more.

Fig. 4. New digital instrumentation 4-8 channel beta model.

A key element of this new digital instrument was the development by John Miles of a new modular digital spectrum analyzer and integral phase detector using a 14 bit, 80 Mbps core. It works from 1 MHz to 20 MHz, needs no antialiasing filter, and uses cross-correlation techniques to achieve a low noise floor. Fig. 5 shows that there are no significant spurs from 1 Hz to 1 kHz in the spectrum analyzer output (red curve). The linearity exceeds 90 dB, which permits the use of tunable sources with higher PM noise than is permissible with the previously used analog double balanced mixer phase detectors.

The new tunable sources use a very low noise 100 MHz quartz oscillator as master clock to drive multiple direct digital synthesis (DDS) chips to generate tunable low PM noise sources for independently interrogating the bacteria of each channel. The 100 MHz oscillator is frequency modulated at 250 Hz to provide the calibration signal for the measurements. The output frequency can be tuned from 1 MHz to 30 MHz with 32-bit resolution to accommodate a wide variety of resonators. Output power is adjustable up to 10 dBm. The isolation between channels exceeds 80 dB. The PM noise of the new source is shown in Fig 5. The spurs at 30, 60, and 70 Hz are due to the reference source. Fig. 5 also shows that the harmonics of the calibration tone at 250 Hz are more than 30 dB below the modulation tone.

A new multi-channel disposable cassette was developed to enable every test to be performed with a clean resonator/sensor, free from possible contamination from previous tests. The cross section of this new cassette, shown in Fig. 6, uses four 14 mm AT-cut, plano-plano, 5 MHz fundamental quartz crystal resonators with gold electrodes, optimized to have minimal temperature dependence near 37°C (normal body temperature).

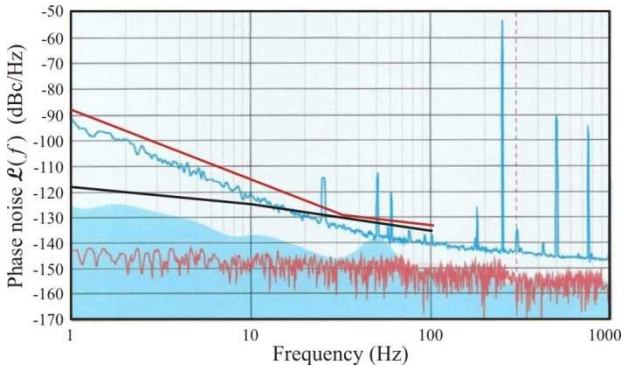


Fig. 5. The red curve shows the noise floor of the new spectrum analyzer + phase detector. The blue curve shows the PM noise ($L(f)$) vs offset frequency f (Hz) of the new DDS sources. The smooth red line shows the maximum PM noise allowable to not increase the detected signal by more than 0.4 dB. The black line shows the PM noise limits if a typical analog mixer were used for the phase detector. The blue region at the bottom shows the uncertainty in the blue curve.

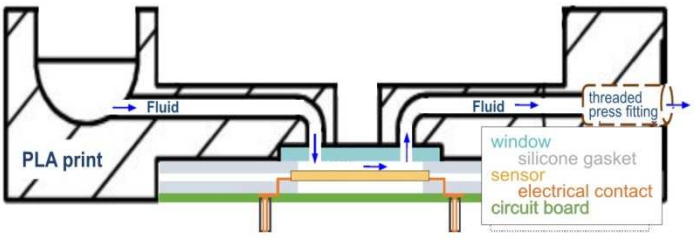


Fig. 6. Cross section of new disposable 3D printed cassette showing the green circuit board on the bottom then the gasket, quartz resonator in gold, fluid, optical window, and cassette top. The reservoir for the antibiotic is top left.

The new design introduces much less stress on the resonator and is much smaller than previous commercial designs, yielding more consistent results at less than 2 % of the cost. Yields of fully performing 4 channels cassettes are 100 % compared to roughly 44% for the original single channel commercial modules.

The base for the cassette contains the low noise analog amplifiers for detecting the added PM noise of the cells and the controller for heaters keeping the disposable cassette near 37°C. The input impedance of the amplifiers is matched to the series resistance of the resonators and includes the capacitance compensation path.

Fig. 7 shows the ultimate resolution of the entire system with the resonator replaced by its series resistance: $L(1\text{ Hz})$ is less than 140 dBc/Hz from 1 Hz to 1 kHz. The lowest-noise resonators are found to be within several dB of this performance. There are no detectable spurs in the range from 1 Hz to 100 Hz. The floor of the brown curve is limited by Johnson noise in the signal and 3 dB noise figure of the amplifiers. Channel to channel electrical isolation exceeds 60 dB. The lack of spurs from 1 to 100 Hz in

the signals shown in Fig. 7 means that the integrals of PM, which were limited to 4-20 Hz in the old analog system, can be extended to 1-100 Hz, which increases the signal and improves the confidence intervals.

The bacteria to be tested are centrifugally separated from clinical UTI samples, without culturing, and introduced onto parallel sensors using a multichannel syringe pump, which is turned off during measurements to minimize acoustic noise.

A new analysis program automatically adjusts the frequency of the DDS probe source for each channel to be very close to the center of the resonance, runs the calibrations for each run, and computes the power spectral density of the measured PM noise. It then scales the data to account for the initial calibration and integrates the PM noise minus the background (without cells) over a selected frequency range to present a single number representative of the added PM noise of each channel. It is then easy to plot this integrated PM value as a function of time to observe the response of the bacteria under test to exposure to multiple antibiotics relative to that of a control channel without antibiotic, in near real time. The entire process for collection, calibration, introduction of the antibiotic and observation of the response takes less than 2 hours.

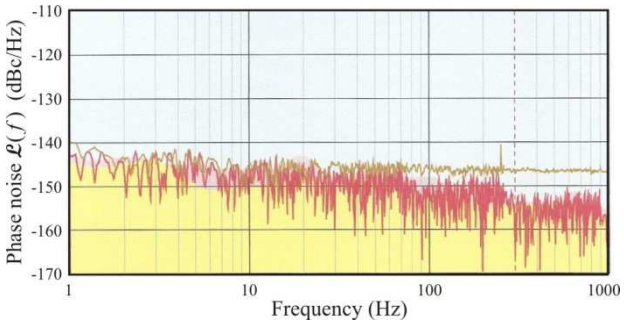


Fig. 7. The brown curve shows typical 5 minute PM noise floor of the amplifiers with a resistor in place of resonator. This is compared to the 5-minute noise floor of the spectrum analyzer + phase detector (red curve from Fig. 6).

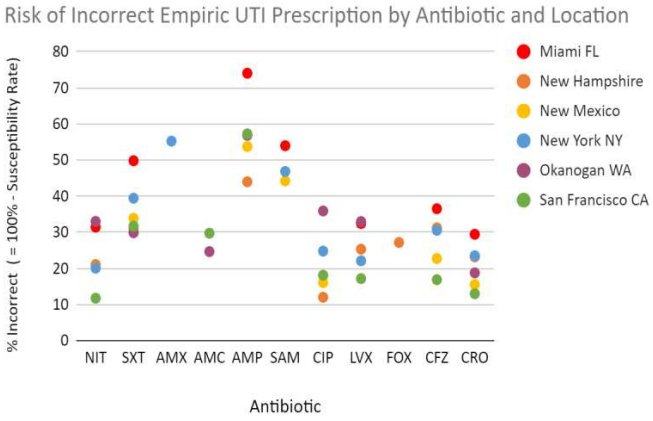
First results with this improved and expanded system show the same form of time-dependent response of *E. coli* to ampicillin as seen previously with the component system and commercial cassettes. After 30 minutes of exposure to 0.1 mg/L ampicillin, treated sensors showed roughly 5-fold lower noise than the control sensor, allowing clear interpretation of susceptibility. The new desktop system can collect over a frequency range of 1 to 100 Hz compared to 4-20 Hz for the analog system. This wider frequency range will facilitate a more accurate analysis of the frequency dependence of the PM noise spectra of the various bacteria.

IV. APPLICATION TO THE CLINICAL LABORTAORY

As mentioned earlier, standard UTI treatment is empiric: health care professionals prescribe an antibiotic as a first guess, then follow up with testing to determine the pathogen and its susceptibility to various antibiotics. If the infection is resistant to the initial antibiotic, the test informs a new prescription for an effective antibiotic. This process takes about 20 minutes to collect the sample and indicate potential infection, one day to grow and count pathogens from the sample, and then another day to monitor pathogen growth in the presence of antibiotics.

The approach described here is one of many efforts to reduce the two days of testing lag time to the point where initial prescription decisions can be made based upon susceptibility data. While empiric prescriptions have served many patients well in the past, rising incidence of antibiotic resistance among UTI pathogens is making this practice less and less practical. Antimicrobial resistance varies by antibiotic, by pathogen, and by geographic location. Physicians rely upon antibiograms, which report recent antimicrobial susceptibility test results, to guide their empiric prescriptions. MPD compiled data from a number of urine antibiograms published by hospital systems across the United States to evaluate the likelihood that a physician is empirically prescribing the wrong drug to a resistant UTI. Fig. 8 shows UTI resistance rates varying from roughly 10 to 70%. Some drugs, like ampicillin, have fallen out of use because the resistance rate is so high. All front line UTI drugs run a significant risk of being ineffective against a resistant infection, thereby failing patients and exacerbating the problem of antimicrobial resistance.

The MPD approach to faster antimicrobial susceptibility testing bypasses the delays needed for pathogen growth and identification in favor of a direct phenotypic measurement of bacterial response to various antibiotics. With no species identification of bacteria, time to prescribe an effective antibiotic reduces to less than 2 hours. However, this can lead to some errors arising from the variety of UTI pathogens, their concentration-dependent responses to antibiotics, and their *in vivo* behavior. An analysis of the distribution of UTI bacteria measured at the Denver Health laboratory in 2017 shows that 87.9 % were *Enterobacteriales,* which includes *E. coli* and *Klebsiella spp.*, and 1.4 % were *Pseudomonas spp.*, both of which stain Gram negative. 6.2 % were *Enterococcus spp.* and 4.1% were *Staphylococcus spp.*, both of which stain Gram positive. Using this distribution of organisms and published data [11] for susceptibility and resistance (*i.e.*, minimum inhibitory concentration distributions for each species-antibiotic combination), testing error rates were calculated to evaluate the performance of the MPD method as applied to UTI clinical microbiology.



AMX Amoxicillin
AMC Amoxicillin-clavulanic acid
AMP Ampicillin
SAM Ampicillin-sulbactam
CFZ Cefazolin
SXT Trimethoprim-sulfamethoxazole
CRO Ceftriaxone
FOX Cefoxitin
CIP Ciprofloxacin
LVX Levofloxacin
NIT Nitrofurantoin

Fig, 8. Risk of incorrect empiric UTI prescription by antibiotic and location in the USA [4-9].

Error rates are classified using the FDA framework [12] as Very Major (VMJ), Major (MAJ), or Minor (MIN). Briefly, a VMJ error misclassifies resistance as susceptibility; a MAJ error misclassifies susceptibility as resistance; and a MIN error misclassifies an intermediate result. An incorrect empiric description made by a physician in the absence of test data would effectively be a VMJ error.

The calculated MPD error rates in Table 1, with VMJ up to 5 - 6 % for some antibiotics but 0 % for others, show great improvement over the 10 to 70 % error potential of empiric prescriptions. However, current FDA approval framework requires VMJ under 3 %. The calculated VMJ errors are driven primarily by *Enterococcus spp.*, not because the test fails, but due to a known discrepancy where *in vitro* susceptibility for *Enterococcus spp.* against trimethoprim/sulfamethoxazole and cefoxitin does not correspond to *in vivo* efficacy for treating UTIs [13]. In standard clinical practice, test results showing *Enterococcus spp.* susceptibility against these drugs are not reported to physicians, and this filtering can only be done with bacterial identification. With these considerations in mind, it becomes clear that the MPD approach will be most powerful when combined with rapid bacterial identification. Identification could be as simple as gram-staining, which would filter out the gram positive sources of error while providing a valid test for the gram negative majority of infections, or as involved as full species identification by an orthogonal method. There is also an intriguing possibility that the PM noise spectra collected in the MPD test can ascertain species identification based on the hypothesis that each species has a characteristic mechanical signature that manifests in the shape of the PM spectra.

	MPD	% error		
	test conc.	*Species-agnostic*		
	mg/L	VMJ	MAJ	MIN
Nitrofurantoin	32	0	0	4.3
Trimethoprim/Sulfamethoxazole	2/38	4.8	0	0
Amoxicillin/K Clavulanate	8	0.1	0	7.0
Levofloxacin	1	0	1.4	2.5
Cefoxitin	8	6.2	0	2.6
Ampicillin	8	0	0	2.3

Table 1. Calculated error rates for MPD 2-hour susceptibility testing without pathogenic species identification.

V. CONCLUSION

Induced PM noise on a resonator is a powerful new tool for measuring the efficacy of antibiotics for the treatment of *E. Coli* infections, which represent approximately 60 - 90 % of UTI pathogens.

A new desktop instrument for making these measurements has been implemented using digital techniques, greatly reducing the size, weight, and cost over the previous analog approach. A 3D-printed multichannel disposable cassette was developed which reduced the cost by 98 % and the size by about 80 % over conventional QCM measurement modules. The acceptance rate for a 4-channel disposable cassette is now 100 %.

Calculated error rates for a species-agnostic induced PM noise susceptibility test show a large improvement over the standard practice of empiric UTI prescriptions, where the risk of the wrong prescription is roughly 10 - 70 %. However, they do not fall below the 3 % FDA limit required for approval. Further work is needed to include rapid bacterial identification and to optimize the test for the clinical microbiology laboratory. MPD actively seeks to transfer this foundational work to an organization with significant research resources and the drive to realize the full impact of this unique biophysical measurement approach on microbiological measurements and global health.

VI. REFERENCES

[1] W. L. Johnson, D. C. France, N. S. Rentz, W. T. Cordell and F. L. Walls, "Sensing bacterial vibrations and early response of antibiotics with phase noise of a resonant crystal", Scientific Reports, 7:12138 (2017). [2] A. L. Flores-Mirales et al., "Urinary tract infections: epidemiology, mechanisms of infection and treatment options,"Nat Rev Microbiol. May; 13(5): 269–284. (2015).

[3] N. M. Dreger, et al., "Urosepsis—Etiology, Diagnosis, and Treatment," Dtsch Arztebl Int. Dec; 112(49): 837–848. (2015).

[4] Miller School of Medicine, Jackson Health System Antibiograms (University of Miami, FL, 2021). http://www.ugotabug.med.miami.edu/jhs-antimicrobial-stewardship-program/jhs-antibiograms

[5] New Hampshire Dept. of Health and Human Services, 2017 State Antibiogram & Implications for Antibiotic Prescribing (State of New Hampshire, 2018). https://www.dhhs.nh.gov/dphs/cdcs/hai/documents/antibiogram-sum-2017.pdf

[6] University of New Mexico Hospital ED January 1 – December 31, 2017, Inpatient Antibiogram (U. New Mexico, Albuquerque, 2018). https://hospitals.health.unm.edu

[7] New York City Health Department, New York City Antibiogram (New York City, 2021). https://www1.nyc.gov/site/doh/providers/resources/antibiogram.page

[8] Three Rivers Hospital, Antimicrobic Susceptibility Report from 01/01/18 thru 12/31/18, (Three Rivers Hospital, Brewster, 2019). https://www.doh.wa.gov/Portals/1/Documents/5000/Antibiogram-ThreeRivers2018.pdf

[9] Zuckerberg San Francisco General Hospital and Trauma Center, Community Health Nework Antimicrobial Susceptibility Studies (Zuckerberg San Francisco General Hospital and Trauma Center, San Francisco, 2019). https://idmp.ucsf.edu/sites/g/files/tkssra4251/f/wysiwyg/ZSFG%20Antibiogram%202018.pdf

[10] P. Dadgostar, "Antimicrobial Resistance: Implications and Costs," Infection and Drug Resistance, 12: 3903 (2019). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6929930/#CIT0008

[11] European Committee on Antimicrobial Susceptibility Testing, Antimicrobial wild type distributions of microorganisms (EUCAST, 2021). https://mic.eucast.org/

[12] Center for Devices and Radiological Health, Antimicrobial Susceptibility Test (AST) Systems - Class II Special Controls Guidance for Industry and FDA (FDA, 2009). https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/antimicrobial-susceptibility-test-ast-systems-class-ii-special-controls-guidance-industry-and-fda

[13] Clinical and Laboratory Standards Institute, Antimicrobial Susceptibility Testing Standards M02, M07, and M11, CLSI Supplement for Global Application M100, 30th ed. (CLSI, 2020).