Environmental Stressors Triggering Antibiotic Resistance in Bacteria

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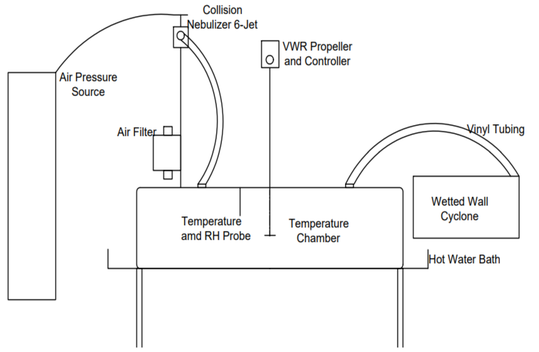
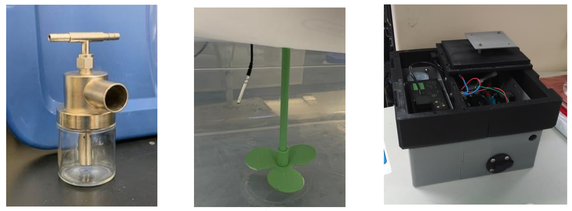
Methodology

Aerosolization:





A. B. C.

Figure 2. Parts of the setup.
(A) Collison Nebulizer 6-Jet atomizes liquid cultures
(B) VWR Propeller with a constant rotation of 80 rpm
(C) Wetted Wall Cyclone (WWC) collects aerosols at 100 L/min

Culturable Counts:

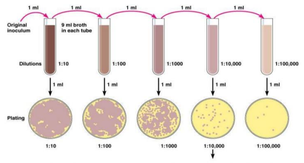


Figure 3. Serial Dilution Plating.
Samples were diluted 10,000x for nebulized and stock E. coli MG 1655 fresh mid-log samples. Aerosol samples were diluted 10x.
The dilutions were plated on Tryptic Soy Agar plates and incubated overnight at 37°C for culturable counts.

Antimicrobial Testing:

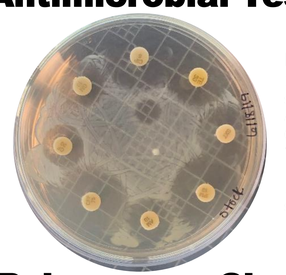


Figure 4. Kirby Bauer Susceptibility test .
Discs with antibiotics were placed on plates of E.coli from stock, nebulized liquid and collected aerosol samples. Antibiotics consisted of Ampicillin (AM), Cefoperazone (CFP), Cephalothin (CF), Gentamycin (GM), Ciprofloxacin (CIP), Tetracycline (TE), Sulfamethoxazole trimethoprim(SXT), and Imipenem(IPM) .
Larger zone of inhibition indicates that the bacteria is susceptible, smaller zone shows resistance.

Polymerase Chain Reaction:

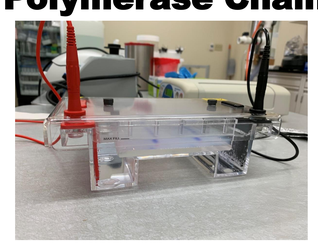


Figure 5. DNA Gel Electrophoresis was used to visualize the specific ARGs amplicons. PCR was used to amplify specific regions of DNA that coded for Antibiotic Resistant Genes (ARGs)
Polymerase Chain Reaction (PCR) was used to visualize the specific ARGs amplicons.

Objective

Is our HVAC system triggering antimicrobial resistance in airborne bacteria?

After months of use an air filter can only filter 70% of particles approximately 3 µm or more.

Which means bacteria can still escape into indoor air.

Humans can inhale particles ≤10 µm in size making it easy for toxic aerosols to enter the human body. What is in the air and how can air effect our health globally? Antibiotic resistance is the cause of 2 million infections and 23000 deaths annually in the US alone (CDC). Multidrug resistant bacteria are the leading cause of lower respiratory infection, morbidity and mortality globally. This study focuses on how air movement, aerosolization time, temperature, and relative humidity can effect the antibiotic resistance of E. coli, a close relative to multidrug resistant pathogens.

Results

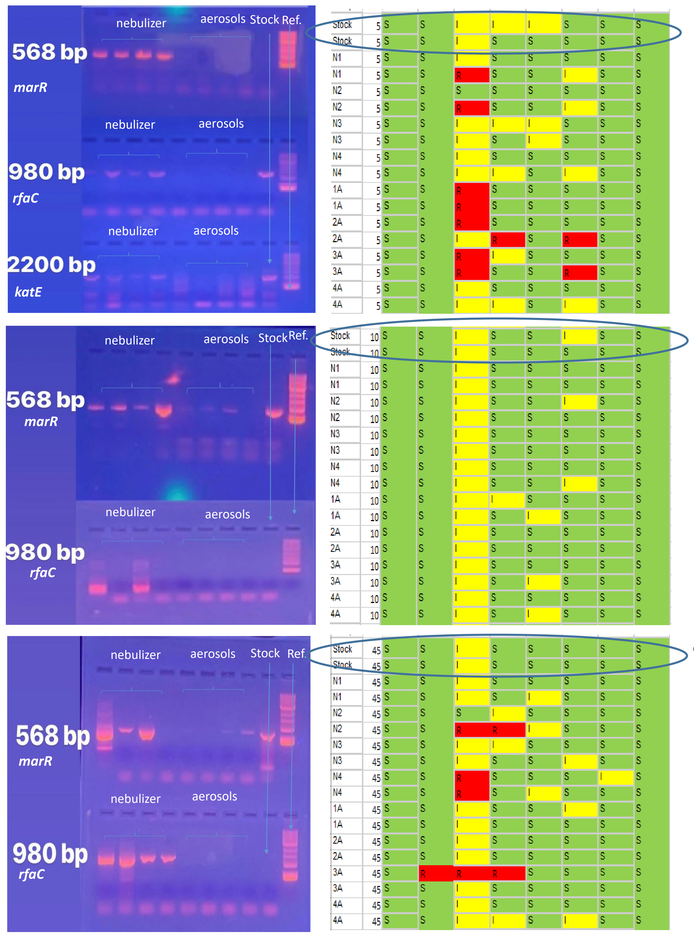


Figure 6. Resistance results after 5, 15 and 45-minute aerosolization. (a) Amplicons of the *marR* and *rfaC* ARGs. (b) Heat map showing bacteria that are Resistant (red), Intermediate (yellow) and Susceptible (green) to the antibiotic (c) Bar charts
All the three ARGs are present in the aerosolized bacteria

Environmental Conditions

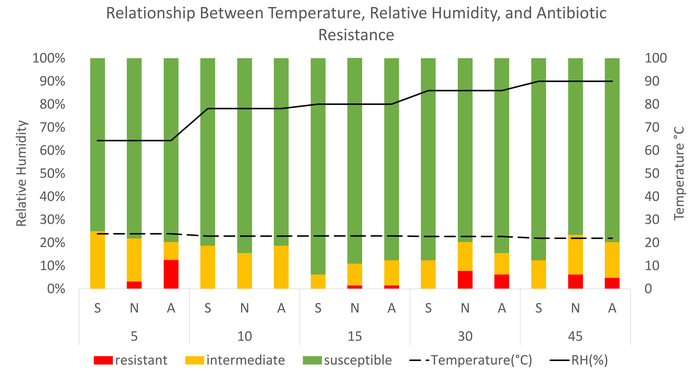
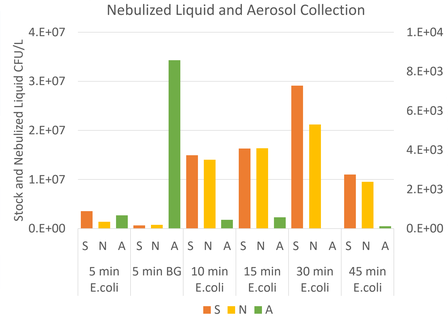


Figure 7. The Effect Environmental Conditions on the Development of Resistance. Stock(S), Nebulized Liquid (N), Collected Aerosol Sample (A).

Sampling Time	Avg. Droplet Diameter, mm
5	1.07147
10	1.09762
15	1.08721
30	1.06342
45	1.08763



a b

Figure 8. Correlation between culturability and aerosol droplet size.
(a)The average CFU count of *E. coli* stock, nebulized samples, and collected aerosols at room temperature and 37°C. The amount of colony forming units (CFUs) were counted for each sample and the average was taken of each sample type, stock(S), nebulizer(N), and collected aerosol(A) samples.
(b) The average droplet size

Discussion

E. coli cells show the ability to express resistant genes and adapt to the environment. During the 5-minute nebulization the *E. coli* became stressed by the change of conditions from 37°C incubation to 20 psi of pressure at room temperature.

- The bacteria aerosolized for 5 min expressed the most resistance.
- The expression of resistance decreased during 10, and 15-minutes possibly due to increased droplet size that may have provided a protective environment.
- Nebulization for 30 or 45 minutes led to the development of resistance likely due to osmotic stress and desiccation.
- During aerosolization at 37°C, higher CFU values were detected compared to the 25°C counts.
- **The stock *E. coli* culture never showed resistance until nebulized or aerosolized.**

In summary, a correlation was found between extended aerosolization times and antibiotic resistance.