The Effect of Different Blood Pressure, Diabetic and Nicotine based Medications on the K-12 Strains of *E. coli* ATCC 4157

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Abstract

The purpose of this project was to discover how the different types of medicines affected the growth of *E. coli* bacteria and whether these results were applicable to different species in the gut microbiome. The effect of the type of medicine on the stress of *E. coli* was determined by a scale of 0-3, 0 being the no growth and 3 being 100% growth. Amlodipine and 0.6% nicotine had level 0 growth in the petri dishes. Metformin and Tylenol had growth equivalent to level 2 culture. These medications may potentially aid in amyloid formation. Losartan and Ibuprofen had level 3 growth with heavily stressed bacteria. Losartan also showed stressed *E. coli* but the Ibuprofen colonies were twice as long as those of the losartan. This might mean that with the same dose, ibuprofen might have more drastic effects than Losartan. 0.3% nicotine had a level 1 growth pattern, but had heavily stressed bacteria. This may also contribute to amyloid formation in the brain. The control had lots of growth, but no stressed bacteria, so it was given a rating of level 3. The BLAST alignment of the csgB and agfB showed 91% similarity (fig. 7), meaning that these medicines may have the same effect on Salmonella bacteria, and possibly other gut microbiome species.

The results showed that the hypothesis was supported and there were some striking results. Ibuprofen had incredulous stress and heavily filamented bacteria. Group 0.6% nicotine had no colonies growing in the petri dish due to possible growth inhibition and 0.3% had minimal growth and all of the bacteria was heavily stressed. The blast alignment of the csgB from *E. coli* and afgB from *Salmonella Typhimurium* protein shows a 91% similarity, which means that the effects of the medicines on *E. coli* bacteria could have a similar effect on *Salmonella typhimurium* and other bacteria species.

Background Research

Neurodegenerative diseases are diseases that affect the neurons in the brain or peripheral nervous system that ultimately die due to gradual or sudden loss of function. Neurons are the primary component of the brain and the spinal cord and form our Nervous system. The neurons however, unlike the other cells in our body, don't regenerate and cannot be replaced by our body. However, neurodegenerative diseases are incurable and extremely debilitating and could have incredibly negative effects on someone. About 60,000 Americans are diagnosed with Parkinson's disease every year (Parkinson's News Today). According to NIH, an estimated 5.4 million Americans currently have Alzheimer's and an estimated 93 thousand and over will get Parkinson's by 2020.

In Europe alone, "The Senior Time bomb" (4) shows that over 16% of the population is above 65 years of age and that figure is expected to rise to 25%. Dementia is one of the major causes of this bomb and annually it costs 130 billion pounds per annum to care for such people which makes this one of the leading social and medical problems in EU Society.

With an increase of Neurodegenerative diseases like Alzheimer's, Parkinson's, Frontotemporal Lobar degeneration, dementia, Huntington's, spinocerebellar ataxia, etc. the question that comes to mind is that is there a common pathway for the occurrence of these neuronal diseases. According to Walker et.al, (1) all of these diseases have a misfolded protein with amyloid polymer structure that behaves like a Prion, and is related to sterile cerebral inflammation. Although the misfolded proteins for all of the above mentioned diseases are different, the biophysical properties of the aggregates are conserved (Walker, 2016). With efforts to extend the life span for all using better technology and medications, the incidence of these neurodegenerative diseases are increasing with age. Scientists have recognized that the need to understand these diseases better is of utmost importance and that both genetic and environmental conditions have a role in contributing to the development of these diseases.

Among the environmental conditions, internal and external environmental conditions like our daily activities, the food we eat, the amount of regular exercises etc, produces a lot of stress on our bodies. Added to it the genetic factors are also important factors that controls gene expression and hence are related to genetic diseases.

There are about 300 - 500 species of bacteria that live in one's gut, containing about two million genes in all. The environment of bacteria in the gut is called microbiome or microbiota, which is different in everyone's guts (Your Gut Bacteria and Your Health). There are many ways in which the bacteria in one's gut maintains the overall health of their body. According to Hao Wang et al., they can "train the immune system, prevent the growth of pathogenic bacteria, regulate the gut development, maintain epithelial integrity, and shape the neuronal development." Our microbiome, which is around 10^14 bacteria which is 10 times more than the amount of cells in a human body. Some of the dominant Genera of bacteria that colonizes the human gut are Clostridium, Bifidobacterium, Peptpcoccus, Ruminococcus, Eubacterium, Bacteroides, Fusobacterium etc. (Zhang, 2015). The composition of the microbiome is influenced by both endogenous and exogenous factors like diet, age, origin and also genetics. Lactobacillus plantarum, a gut bacteria regulates tight junctions in the cells to protect the cells from chemicals that causes a disruption of epithelial lining of the gut (Karczewski, 2010). With compromise on the epithelial integrity, gut bacteria, toxins, undigested food passes into the blood through the gut walls causing something called Leaky gut syndrome.

Escherichia coli or *E. coli*, another common bacteria, also lives inside humans' and animals' intestines. The harmless species that usually live in the gut produce vitamin K and helps digest food. Some species of *E. coli* are pathogenic and can cause infections and diseases which come from contaminated water and food, and then makes its way into people's and animals' guts (GMFH). According to Rachel Ross from LiveScience, the bacteria that cause severe infections produce a toxin called Shiga. Therefore, they are named Shiga-toxin-producing *E. coli* or STEC. These species of *E. coli* is usually found in contaminated water, joces, fresh products, manure, and foods like cheese and meat. Also many scientists report that sometimes not washing hands after coming in contact with animals can play a huge role in the spread of pathogenic *E. coli*.

Enterotoxigenic *E. coli* or ETEC is a type of pathogenic *E. coli* that causes stomach problems including traveler's diarrhea and diarrhea related diseases (*E. coli*). LiveScience reports that "Worldwide, ETEC is estimated to infect at least 280 million to 400 million children under age 5 per year, primarily in developing countries." They infer that many children are victims because of their lack of natural immunity and a strong immune system, resulting from their young age. Sometimes infections caused by STEC can cause Hemolytic Uremic Syndrome (HUS), which causes destruction of red blood cells, eventually leading to kidney failure.

ETEC, a pathogenic species of *E. coli* from Global Health Primer causes urinary and digestive issues. They are relatively found in more contaminated or unsanitary areas, including dirty water, food, or fresh produce. Younger children and older adults are more likely to get infected by STEC than other people in different age groups because of their weakened immune systems. According to LiveScience, about 5 - 10% of people get HUS from STEC infections, which is also responsible for 90% of urinary tract infections. Usually, people experience less frequent times using the bathroom and lethargy. Overall, these symptoms are severe and should be treated immediately, for there are risks of severe diseases and disorders.

Tying back to Parkinson's disease, Science News reports that Parkinson's disease has a direct correlation to one's gut microbiota, including *E. coli*. Scientists infer that *E. coli* may play a significant role in plaque formation in the gut during stress, that might travel up to the brain via different pathways to cause neurodegenerative diseases. Hence, the need to look into the plague formation feature in E-coli becomes a very important research to investigate in as a way to find a connection between the gut and the brain.

Experimental Design:

IV: The type of medication

DV: Stress and Growth of *E. coli*

Constants: the strains of K-12 *E. coli* used

- the temperature of the incubator
- the kind of media used to grow the bacteria
- the amount of time used to grow the bacteria
- the Congo red stain used to stain all the colonies after growth
- the microscope and the magnification (100X under oil immersion) used

Control: We had a negative control group because there was nothing added; it was just pure culture.

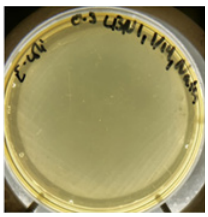
Hypothesis: If the different types of medicines and products people use every day like metformin, ibuprofen, amlodipine, losartan, tylenol, and nicotine affects the length of the *E. coli*, then the ibuprofen, will show the most stressed bacteria followed 0.6% Nicotine, 0.3% nicotine, Losartan, Amlodipine and Metformin and Tylenol. This will happen because ibuprofen can have catastrophic digestive side effects like different digestive ulcers, which may be related to the stressed bacteria in the gut microbiome (Common Drugs Increase Health Risks).

Sample Size: each bacterial culture trial was repeated 5 times.

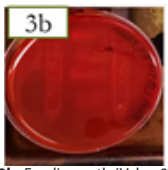
Materials and Methods:

Materials:

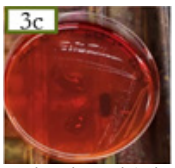
- 1000 mg metformin
- 250 mg losartan
- 25 mg amlodipine
- 1000 mg tylenol
- 1000 mg ibuprofen
- 1 bottle of nicotine (we used blueberry cheesecake flavor)
- 1 bottle of congo red
- 35 petri dishes
- K-12 *E. coli* strains (specifically used for lab purposes)
- Agar media
- Inoculating loop
- 7 erlenmeyer flasks
- Bunsen Burner
- Weigh scale
- Beakers
- Mortar and Pestle



3a. Culture plate with no stain



3b. *E. coli* growth (Value 0)



3c. ⅓rd *E. coli* growth (Value 1)

3d. ⅔rd *E. coli* growth (Value 2)

3e. Full *E. coli* growth (Value 3)

Fig. 3 a through e. Culture plates. 3a is an unstained culture plate. 3b to 3a is stained by Congo red 3b shows no bacterial growth and given a value 0, while 3c shows stressed bacterial growth on 1/3rd of the plate and was given value 1, 3d shows stressed bacterial growth in almost 1/2 of the plate and was given a value 2 and finally 3e showed stressed bacterial growth all over the plate and was given a value of 3.

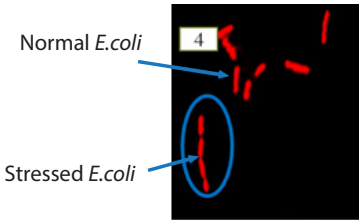


Fig. 4. Showing the difference between stressed and unstressed *E.coli*

Methods:

- The amount of stressed bacteria was determined by staining of the culture plates using Congo Red and a scale of 0-3 was implemented to measure the amount of growth. Congo red is a stain that helps identify filamentous bacteria, see Fig. 3c-e.
- Filamentous bacteria are formed when the *E. coli* bacteria fails to undergo cytokinesis during cell division and form long filaments under stress (fig 4).

Procedures:

Procedures for making the medication plates:

- Pre Measure 5 doses of each experimental treatment: Metformin(M), Losartan(L), Amlodipine (A), Ibuprofen (I), Tylenol (T), 0.3% nicotine (N1) and 0.6% nicotine (N2). Then grind up the solid experimental treatments into a fine powder and label them.
- Measure 30.5 grams of "Luria Bertani" powder onto a clean weigh boat and place the powder into 1 liter of distilled water in a 2 liter erlenmeyer flask with a stir bar (according to the recipe).
- Label the flask with autoclave tape and cover the top opening with aluminum foil
- Place the flask on a hot plate and bring to a boil while stirring on a low stir setting until mixture becomes homogeneous and all of the powder is dissolved.
- Label 6 empty 200 ml flasks with autoclave tape and cover then each with aluminum foil. Label each flask with the different experimental treatments respectively (Metformin(M), Losartan(L), Amlodipine (A), Ibuprofen (I), Tylenol (T), 0.3% nicotine (N1) and 0.6% nicotine (N2).
- Place the flask containing media and smaller flasks into autoclave bins and label each bin with autoclave tape.
- Autoclave all the flasks at a setting for 40 Liter liquid flasks.
- PreLabel 5 Sterile Petri dish Plates for each experimental/control treatment with the treatment type, date, and name
- Wipe downpour area on benchtop with 10% bleach and start a flame with the Bunsen burner to keep the area more steril.
- After autoclaving, cool the media to 48 degrees Celsius and transfer 125 ml into each of the 6 empty flasks aseptically under the Bunsen burner flame using a serological pipet.
- Aseptically mix in the experimental treatments respective to the label on each flask
- From each labeled flask, aseptically measure out 25ml into the appropriately labeled plate using a serological pipet under the Bunsen burner flame
- Once the media had cooled and solidified, flip over plates to prevent excessive condensation (lid facing down)
- The next day, check plates for contamination and ensure there are no bubbles or excessive wetness on the media.
- Take isolated single colonies from a pure bacteria culture and streak each plate fully three times aseptically with a sterile loop.
- Incubate plates upside down in an incubator set at 37 degrees Celsius for at least 24 hours.
- Observe and record results

Data Collection

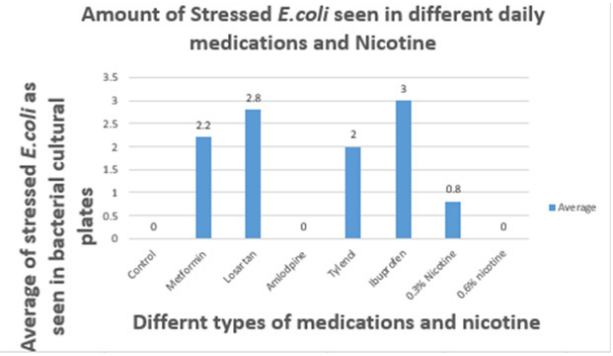
- After observing, take all petri dishes and place them onto a tray. Take out all lids of petri dishes and coat every dish with congo red dye.
- Let the congo red dry for 2-3 minutes and then pour out the excess congo red onto the tray. This way, the bacteria will be easier to see under the microscope.
- Place a slide of bacteria on a slide with 100x oil immersion setting. Take pictures of the *E. coli* bacteria and compare it to the control.

Type of medication	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5
Control	Growth, No stress	Growth, No stress	Growth, No stress	Growth, No stress	Growth, No stress
Metformin	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed
Losartan	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed
Amlodipine	No growth, no stress	No growth, no stress	No growth, no stress	No growth, no stress	No growth, no stress
Tylenol	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed
Ibuprofen	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed	Growth, stressed
0.3% Nicotine	Little growth, Little stress	Little growth, Little stress	Little growth, Little stress	Little growth, Little stress	Little growth, Little stress
0.6% Nicotine	No growth, no stress	No growth, no stress	No growth, no stress	No growth, no stress	No growth, no stress

Table 1: Compares the culture plates based on growth of normal *E. coli* colonies and the ones with filamentous colonies.

Effect of Different Types of Medications on the Growth of stressed *E. coli*						
Type of medications	**Plate 1**	**Plate 2**	**Plate 3**	**Plate 4**	**Plate 5**	**Average**
Control (no medication)	0	0	0	0	0	0
Metformin	2	3	2	2	2	2.2
Losartan	3	3	3	3	2	2.8
Amlodipine	0	0	0	0	0	0
Tylenol	2	2	2	2	2	2
Ibuprofen	3	3	3	3	3	3
0.3% Nicotine	0	1	1	1	1	0.8
0.6% Nicotine	0	0	0	0	0	0

Table 2: Shows the amount of stressed *E. coli* growing under the adult doses of various medications as seen by staining it with Congo Red stain. The numbering 0 to 3 pertains to 0=no growth, 1= growth in 1/3 of the plates, 2=Growth in 2/3 of the plates and 3= growth in all over the plates.(Please see methods for picture reference).



Graph 1: Showing Amount of stress levels in different medications and Nicotine. 0 = no stress, 1 is 1/3 plate filled with stressed bacteria, 2= 2/3 plate filled with stressed bacteria, 3= 100% plate filled with stressed bacteria. *Look at methods for the scale with visual examples.

- Repeat the above procedures as necessary for each type of bacteria that is to be tested

Statistical Methods:

Proper lab attire and lab coat will be required as well as gloves. The congo red is toxic and lots of caution will be required because we are dealing with bacteria. We can tell if the bacteria is stressed by how much longer it is. Also, all tools should be sterile for the risk of contamination is there for both the scientist and experiment.

Results:

Data Tables: See Tables 1-2 and Graph 1

Data Analysis:

The table and graph shows the stress and growth of the *E. coli* bacteria based on the different types of bacteria.

- Amlodipine and 0.6% nicotine had no growth in the petri dishes because the *E. coli* growth was hampered. As a result the bacteria could not be identified as stressed or not stressed. This might mean that nicotine and amlodipine have a negative influence in the growth of beneficial gut bacteria.
- Metformin and Tylenol had approximately 3/4th of the plates with stressed bacteria. This shows that these medications might have a potential of giving rise to neurodegenerative diseases.
- Losartan and Ibuprofen had 100% of the plates with stressed bacteria. This shows that these medications might have a potential of giving rise to neurodegenerative diseases just like Metformin and Tylenol.
- Losartan also showed stressed *E. coli* but the Ibuprofen colonies were twice as long as those of the losartan. This might mean that with the same adult dosage, ibuprofen might have more drastic side effects than Losartan.
- 0.3% nicotine had a very small amount of growth and had multiple stressed bacteria.
- The control had growth and no stressed bacteria.
- The measurements of the binding and dissociation kinetics of Congo red were taken on *E. coli* strains MC4100, also known as K-12 strain (this strain is in the gut, it is not pathogenic). It was discovered that the binding of Congo red to the curli bacteria depends on the pH (7.4) of the environment

Conclusion:

- Overall, almost all of the medications used showed stressed bacteria, making longer bacterial colonies in almost every group. Some didn't have any colonies because of the amount of stress.
- The hypothesis was "If the different types of medicines and products people use every day, metformin, ibuprofen, amlodipine, losartan, tylenol, and nicotine affects the length of the *E. coli*, then the Ibuprofen will show the most stressed bacteria."
- This hypothesis was supported and refuted because ibuprofen had the longest colonies with filaments, almost four times the original size, proving the amount of stress the bacteria had was incredulous.
- In some ways the hypothesis was refuted because 0.6% nicotine had no colonies growing in the petri dish and 0.3% had minimal growth. This probably happened because nicotine causes several health hazards like an increased risk in gastrointestinal disease, which could stress out the bacteria.

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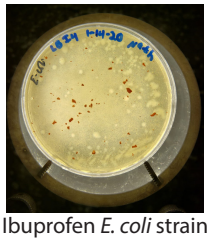
Pictures



Ibuprofen *E. coli* strain