Airborne Threats: Investigating the Effect of Particulate Matter (PM2.5) on the Antibiotic Resistance of *Escherichia coli*

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Abstract

This project investigates a possible correlation between increased air pollution (PM2.5) levels and antibiotic resistance through a controlled experiment using car exhaust to simulate PM2.5. The hypothesis is that if PM2.5 concentration correlates with E. coli antibiotic resistance, then longer car exhaust exposure times will correlate with smaller zones of inhibition (ZoI) and greater bacterial growth. A possible mechanism for increased antibiotic resistance is that particulate matter can facilitate horizontal gene transfer by increasing the permeability of cell membranes and disseminating genes in the air (Zhou et al., 2023). Several E. coli plates were exposed to varying amounts of PM2.5 dictated by varying car run times. Exposed bacteria was then spread on petri dishes divided into quadrants, where antibiotic discs of varying concentrations were placed. Data collection consisted of measuring the ZoI diameter and counting satellite colony growth inside the ZoI. Colonies growing inside the ZoI showed antibiotic resistance, growing even in the presence of antibiotics. Experimentation found a positive correlation between increased PM2.5 and antibiotic resistance because plates with E. coli exposed to more car exhaust had more colonies. Although the association between PM 2.5 and antibiotic resistance has been studied using modeling techniques, this study tests the correlation between the two variables using live microorganisms and aerosolized PM 2.5 in a controlled environment. Findings can be applied when addressing the need to reduce air pollution and show that PM 2.5 may contribute to the antibiotic resistance crisis.

Keywords: **PM2.5, antibiotic resistance, Particulate pollution, Zone of inhibition, Bacterial transformation, PM2.5 concentration, Antibiotic disc**

Introduction

Antibiotic resistance has become a prominent global problem due to several factors, including the lack of establishment of proper protocols. These include improper disposal, usage, and prescription policies. This rising problem affects the health of people all around the world, raising concerns about disease and making treatment much harder. Simultaneously, the concentration of particulate matter (PM2.5) continues to grow, posing a risk to human health. This study aims to bridge two seemingly vast research areas to determine if these problems could be connected experimentally, and even exacerbate their effects.

Literature Review

PM2.5is composed of fine inhalable particles that are generally 2.5 micrometers or smaller and has increased in the atmosphere due to a rise in the usage of fossil fuel-powered vehicles (EPA 2024). This miniscule air pollutant "is responsible for nearly 4 million deaths globally from… heart disease, respiratory infections, chronic lung disease, cancers, preterm births, and other illnesses" (Thangavel, 2023), raising concerns regarding the need to reduce its concentrations in our atmosphere. PM2.5largely consists of emissions from gasoline, oil, diesel fuel, and wood (California Air Resources Board, 2024), which is why car exhaust was used to simulate it in this experiment. As climate change concerns grow, PM2.5 becomes more of a threat to human health, affecting the prevalence of heart and respiratory disease. Although the prevalence of this air pollutant varies, it's still becoming more and more common in virtually every geographic location, making it an important issue to address.

The intersection of these disciplines (PM2.5 pollution and antibiotic resistance) is a rising concept that could make addressing these issues crucial. It is hypothesized that PM2.5 could increase the permeability of cell membranes, advancing transformation through horizontal gene transfer. (Zhencao et al., 2023). This hypothesis is further supported by a study done on the transmission of ARGs through heavy metals (which are another component of PM2.5), which explains that "...Environmental pollutions, such as metal, can accelerate ARG dissemination" (Zhang et al., 2018). This is becoming pivotal in the fight against both climate change and the antibiotic resistance crisis, as both PM2.5levels and improper antibiotic usage levels are rising and are responsible for several deaths, Zhenchao et al. explain that "antibiotic resistance derived from PM2.5 caused an estimated 0·48 million premature deaths corresponding to an annual welfare loss of US $395 billion due to premature deaths" (Zhencao et al., 2023). The following methodology was used to test this in a controlled experiment.

Methodology

This experiment utilized a controlled scientific approach to investigate the effect of PM2.5 particulate matter on the antibiotic resistance of E. coli. An exposure chamber was constructed using a clear plastic container and PVC piping, using a sealant to create an airtight seal. The chamber is attached to the tailpipe of a 2023 Mazda CX-5 to simulate real-world PM2.5 exposure. This chamber was designed from the schematic of the indoor chamber in the Research Center for Eco-Environmental Sciences (Chu et al., 2022), but was simplified using only the critical components of this design, marked in black.

Next, Petri dishes containing E. coli were exposed to PM2.5 within this chamber for either 90 seconds or 5 minutes. To examine the impact of exposure timing, half of the dishes were exposed immediately after plating, while others were exposed after 16 hours of incubation at 36. Exposing the incubated E. coli to PM2.5 was an attempt to visualize its effects on an established E. coli colony, as explained in a study by Lilja Brekke Thorfinnsdottir. "The results were also highly dependent on…pregrown physiological state of the cells" (Thorfinnsdottir et. al., 2023).

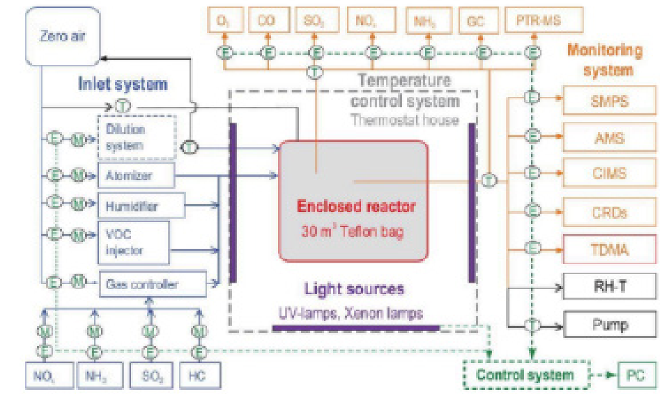

Fig. 1. Schematic for chamber (Chu et al., 2022)

Fig. 2. PM2.5 chamber with lid removed

For each E. coli growth time before exposure (immediately after or after 16 hours of incubation), three dishes were exposed for 90 seconds and 5 minutes. The petri dishes were then incubated inside the airtight chamber for either 20, 40, or 60 minutes (one, two, or three E. coli doubling cycles) at room temperature. This provided more time for the development of potential E. coli antibiotic resistance. Once the E. coli had been exposed, four antibiotic discs with varying concentrations of norfloxacin were placed in each quadrant of the petri dish (the control disc was deionized water). To measure the ZoI of each norfloxacin disc, the Kirby-Bauer test was used, measuring the diameters of the ZoIs and the number of colonies inside the ZoIs.

Results

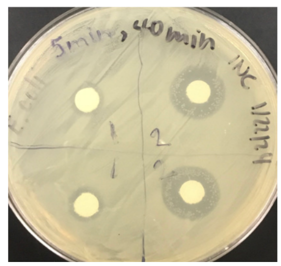

Fig. 3. Trial 8 of the E. coli exposed to car exhaust for 5 minutes. Colonies of mutated bacteria in the zone of inhibition are present.

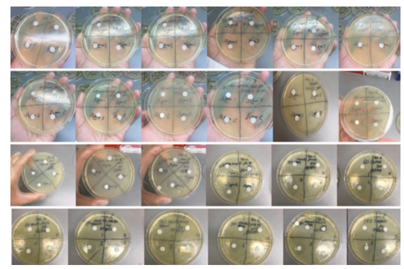

Fig. 4. Depicts plates spread with E. coli grown in liquid media and 4 antibiotic discs with 0, 1, 2 and 5 mg/L of Norfloxacin.

The Effect of Car Exhaust (PM 2.5 Concentration) and Norfloxacin Concentration on Zones of Inhibition on E. coli

Car Exhaust (PM 2.5) Exposure Time	Diameter of Zone of Inhibition (mm)															
	Trials															
	1				2				3				4			
	Norfloxacin Concentration on Disc (mg/L)															
	0	1	2	5	0	1	2	5	0	1	2	5	0	1	2	5
0 sec, 0 min	0	8	14	24	0	14	21	25	0	12	15	23	0	16	22	24
90 sec, Overnight	0	9	19	22	0	14	18	23	0	15	21	24	0	15	22	24
90 sec, Immediate	0	10	15	20	0	15	16	25	0	15	22	25	0	18	21	25
5 min, Overnight	0	14	17	25	0	15	21	24	0	10	14	25	0	15	23	26
5 min, Immediate	0	10	20	25	0	0	0	16	0	10	14	23	0	0	0	15
	Trial 5				**Trial 6**				**Trial 7**				**Trial 8**			
	1mg/L	1mg/L	2mg/L	2mg/L	1mg/L	1mg/L	2mg/L	2mg/L	1mg/L	1mg/L	2mg/L	2mg/L	1mg/L	1mg/L	2mg/L	2mg/L
	10	10	17	15	8	14	15	17	9	6	11	14	16	7	16	17
Diesel Particulate Matter	0	8	10	15	0	12	17	24	0	14	23	26	0	14	24	28

Fig. 5. Depicts diameters of zones of inhibition. There were 4 trials of each Norfloxacin concentration performed, and 6 levels of car exhaust exposure. Note that there are an additional 4 trials for 5-minute, immediate exhaust exposure with 1 and 2 mg/L of Norfloxacin.

The Effect of Car Exhaust (PM 2.5 Concentration) and Norfloxacin Concentration on E. coli Colonies Grown in Zone of Inhibition

Car Exhaust (PM 2.5) Exposure Time	Colonies (number)															
	Trials															
	1				2				3				4			
	Norfloxacin Concentration on Disc (mg/L)															
	0	1	2	5	0	1	2	5	0	1	2	5	0	1	2	5
0 sec, 0 min	0	0	0	0	0	6	5	0	0	0	3	0	0	0	0	0
Diesel Particulate Matter	0	3	11	0	0	0	0	0	0	0	1	0	0	0	0	0
90 sec, Overnight	0	0	1	2	0	41	27	50	0	0	0	0	0	0	0	0
90 sec, Immediate	0	3	14	1	0	2	2	0	0	1	1	0	0	3	5	12
5 min, Overnight	0	11	9	2	0	5	4	0	0	0	0	0	0	0	0	0
5 min, Immediate	0	30	11	0	0	TNTC	TNTC	TNTC	0	5	0	0	0	TNTC	TNTC	TNTC
	Trial 5				Trial 6				Trial 7				Trial 8			
	1mg/L	1mg/L	2mg/L	2mg/L	1mg/L	1mg/L	2mg/L	2mg/L	1mg/L	1mg/L	2mg/L	2mg/L	1mg/L	1mg/L	2mg/L	2mg/L
	25	20	35	30	50	50	46	15	30	15	43	19	41	50	45	50

Fig. 6: Depicts numbers of colonies inside the zones of inhibition. There were 4 trials of each Norfloxacin concentration performed, and 6 levels of car exhaust exposure. Note that there are additional 4 trials for 5-minute, immediate exhaust exposure with 1 and 2 mg/L of Norfloxacin.

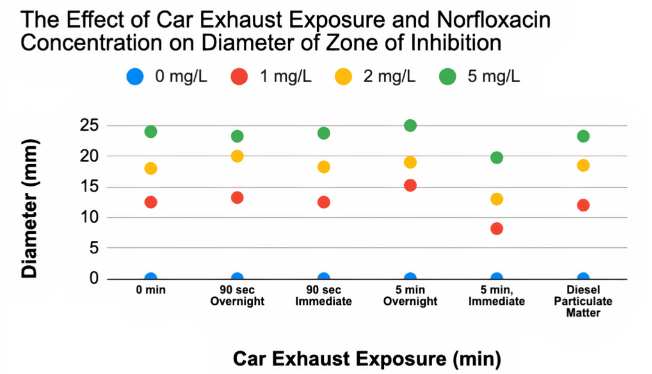

Fig. 7: Depicts means of diameters of the zone of inhibition from the trials shown in Fig. 5. Note the smaller zone of inhibitions for 5 minutes, immediate exposure, indicating lower effectiveness of Norfloxacin. This shows that the mean diameter of the zone of inhibition was lower for the highest car exhaust exposure time.

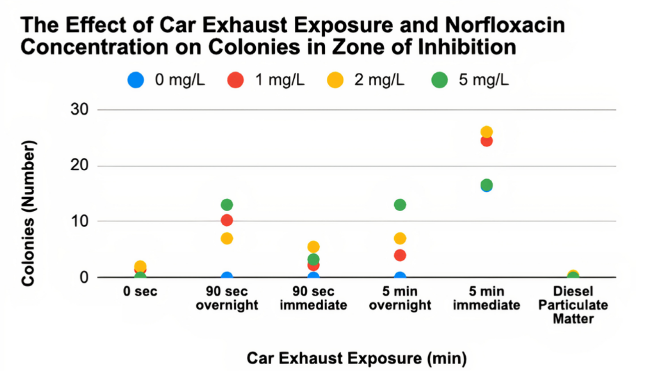

Fig. 8: Depicts means of the number of microbial colonies in the zones of inhibitions from the trials shown in Fig. 6. Microbial growth in the zone of inhibition is present in 5-minute immediate, 5-minute overnight, and 90-second overnight exposure levels.

Discussion

The results of this experiment support a positive correlation between exposure to car exhaust (simulating PM2.5) and antibiotic resistance; shown through a decrease in the mean zone of inhibition and an increase in the number of colonies grown in the zone of inhibition. At 5 minutes of immediate exposure, the mean diameter of the zone of inhibition for 2 mg/L was 20 mm, with 26 colonies in the zone; at 5 min overnight, the diameter was 18 mm with 7 colonies in the zone; and at 90 sec, immediate, the mean diameter was 18 mm with 6 colonies in the zone. This data is compared to 0 minutes, where the mean diameter of the zone of inhibition was 18 mm and there were 2 colonies in the zone. At 2 mg/L, the number of colonies inside the ZoI for the plates exposed to car exhaust (90 sec overnight, 90 sec immediate, 5 min overnight, and 5 min immediate) was significantly higher than the control.

The correlation between PM2.5 and global antibiotic resistance had recently been observed by Zhencao et al. through a fixed-effect panel model that analyzed more than 11.5 million tested isolates of antibiotic-resistant strains. Through their study, the researchers showed, "a consistent association between PM2.5 and aggregate resistance across regions and pathogens, indicating that PM2.5is one of the primary factors driving global antibiotic resistance." Overall, the hypothesis by Zhencao et al. (2023) that increased PM2.5levels are associated with antibiotic resistance because it increases horizontal gene transfer was not fully supported. Although there was a decrease in the diameter of the zones of inhibition and an increase in the number of mutated colonies, it cannot be proved whether the E. coli that grew in the zone was transformed or mutated randomly.

Conclusion

Overall, the positive correlation between increased PM2.5 concentration and antibiotic resistance shows that the increase in car exhaust exposure most likely increased the mutation rate of E. coli in the presence of Norfloxacin because there were significantly more mutated colonies in the plates exposed to car exhaust compared to the control.

It cannot be concluded that transformation (meaning uptake of foreign DNA) was increased by PM2.5, and further research is needed to understand the mechanisms behind the results. However, this study answers the research question by utilizing a controlled experiment to show that antibiotic resistance increases with increased PM2.5 concentrations. Although the association between PM2.5 and antibiotic resistance has been studied using modeling techniques and a dataset of antibiotic resistance over several years, this study tests the correlation between the two variables using live microorganisms and aerosolized PM2.5in a controlled environment. This experiment's results can guide further works related to medicine, the environment, and other topics, as PM2.5 has been identified as an important factor in increasing antibiotic resistance.

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