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Mutagenicity of Bulk, Aqueous and Organic Partitions of Air Particulate Matter in Differentially Ventilated Wards in a Public Urban Hospital

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ABSTRACT

The hospital environment requires indoor air quality conducive to the recovery of patients with poor health. Low indoor air quality is associated with an increased incidence of respiratory tract diseases and the development of cancer. This study investigated the mutagenicity of air particulate matter soluble in bulk, aqueous, and organic partitions collected from naturally and mechanically ventilated wards in the hospital environment through the Ames test and the mutagenicity testing with the D7 strain of *Saccharomyces cerevisae*. Bulk, aqueous, and organic fractions of air particulate matter at maximum (100% concentration), 10% concentration, and 1% were found to be mutagenic with both the Ames test ($p < 0.05$) and mutagenicity testing with D7 strain of *S. cerevisae* ($p < 0.05$). The Ames test suggested slight dominance of the aqueous phase-soluble mutagens in naturally ventilated wards ($p < 0.05$), and a more balanced mix of aqueous and organic phase mutagens in mechanically ventilated wards. Mutagenicity testing with the D7 strain of *S. cerevisae* showed no significant differences between the naturally and mechanically ventilated wards ($p > 0.05$), but showed the relative dominance of the organic phase-soluble mutagens over the other fractions ($p < 0.05$). Few other studies have compared naturally and mechanically ventilated wards through the lens of potential effect on the mutagenic activity of air particulate matter, but more understanding in this area is important in moving towards the development and implementation of policies to optimize ventilation systems for the health and safety of hospital staff and patients. Albeit coming from the study of concentrated air particulate matter samples, the mere presence of these mutagens in the air of the hospital highlights the importance of monitoring their quality and quantity such that they do not become concentrated enough to induce mutation-related etiologies of disease such as cancer.

INTRODUCTION

Poor indoor air quality is associated with an increased incidence of respiratory tract diseases, cardiovascular diseases, and cancer (Perez-Padilla et al. 2010). Indoor air pollution is split into two major categories: bioaerosols and volatile organic compounds. Air particulate matter that comes from living things such as pollen and seeds from plants, spores from fungi, bacteria and their endotoxins, animal dander, and even expelled breath or scattered latex from humans are considered bioaerosols. Volatile organic compounds usually refer to polycyclic aromatic hydrocarbons (PAHs), which can be found attached to air particulate matter, both indoors and outdoors, and are known to have mutagenic and carcinogenic effects (El-Sharkawy & Noweir 2014, Loh et al. 2007).

Generally, indoor air pollution comes from outdoor air, cigarette smoke, activities such as cooking and cleaning, and emissions from appliances such as air conditioners and fax machines (Apte & Salvi 2016). This means that how a space is ventilated determines the characteristics of the air particulate matter contained within it. Naturally ventilated spaces have the benefit of higher turnover rate of air in a room, quickly dispersing any existing pathogens and harmful particles, but exposing people to new pathogens and particles unfiltered. Mechanically ventilated spaces have the benefit of filtration of the air that enters the room, but with both a slow turnover rate and an added source of pollutant: the air conditioner and/or filter when contaminated (Hobday & Dancer 2013). Both the types of ventilation can be found in the hospital setting.

The hospital requires indoor air quality conducive to the recovery of patients with poor health. These vulnerable pop-

ulations are typically more susceptible to the development of diseases and potentially harmful effects of pollution in their environment. Studying the air quality and characteristics of the air particulate matter according to the type of ventilation has implications on the development of policies and practices that may improve the indoor air quality in the hospital, and thus, reduce any disease risks associated with poor air quality.

The Ames test in *Salmonella typhimurium* is the gold standard for mutagenicity testing. A variety of strains exist to test for the induction of different mutations with the repair systems for the genetic material removed. In the absence of S9 activation, substances are tested for their mutagenic activity in their entirety, not undergoing any sort of metabolism or fragmentation (Mortelmans & Zieger 2000). Mutagenicity testing using the D7 strain of *Saccharomyces cerevisae*, a strain developed and described by Zimmerman (1975), is less mainstream, but being a eukaryotic system, possesses the natural ability to metabolize substances without any additional enzymes to the media and with a single strain tests for three types of mutagenic activity alongside their respective DNA repair mechanisms. This model provides a good contrast to the prokaryotic, non-metabolizing, non-repairing *S. typhimurium* that is the most well-known and widely-used methodology in mutagenicity testing.

MATERIALS AND METHODS

Air Sample Collection and Processing

Air sampling was performed for seventy-two hours (72 hours) in four different hospital wards, 2 naturally ventilated and 2 mechanically ventilated, with roughly the same floor area per ward. During the collection period, both the wards were filled to capacity. The naturally ventilated wards contained about 70 people on average (30 beds for patients, all of whom had at least 1 companion with them constantly and 8-12 health professionals on duty). The mechanically ventilated wards were both ventilated by the same type of air conditioner set-ups and contained an average of about 30 people during the collection period (20 beds for patients, no patient companions as per hospital policy, and a team of 8-12 health professionals).

The air collection set-up was a chamber containing sterile phosphate buffer solution (PBS) attached to an air collection pump placed at a height of about 1.5 meters (average height at which humans breathe) inside each ward. Immediately following collection, the suspension was filtered through Whatman filter paper (pore size 0.45 μm), and fractionated into aqueous and organic phases. A portion of the bulk phase was also kept to be run alongside the fractionated components. Samples were stored at 4 degrees Celsius. For both, the Ames test and the mutagenicity testing with the D7 strain of *S. cerevisae*, samples were serially diluted prior to testing. Three concentrations of each fractionated sample were used: undiluted or 100% concentrated (100% conc.), 10% concentrated (10% conc.), and 1% concentrated (1% conc.).

Ames Test

Salmonella typhimurium strain TA100 was procured from Dr. Keichi Sugiyama of the Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences (Tokyo, Japan). The TA100 strain detects base-pair substitutions on the *hisG* gene for histidine synthesis without the benefit of any type of repair system for DNA damage (Maron & Ames 1983). Ames test for mutagenicity traditional pour plate method was performed according to established protocols (Ames 1975, Maron & Ames 1983, Mortelmans & Zieger 2000). S9 activation was not used. Bacterial cells were incubated with shaking for 18 hours at 37 degrees Celsius in LB broth with ampicillin prior to their exposure to the air particulate matter samples to ensure that the bacteria were at the log phase during the exposure. Following a two hour period of exposure to samples at 37 degrees Celsius, sample was washed off. A positive control of sodium azide dissolved in sterile PBS (10 μg/mL) and negative control of sterile PBS alone were run alongside the samples. All the assays were run in triplicate.

Mutagenicity Testing in the D7 Strain of *S. cerevisae*

The D7 strain of *Saccharomyces cerevisae* was a gift from Dr. Pamela Marshall of the School of Mathematical and Natural Sciences, New College of Interdisciplinary Arts and Sciences, Arizona State University (West campus, Arizona, USA). Isolation and preparation of the D7 strain were performed according to previously validated and published protocol (Marshall 2007, Zimmerman 1975). Three types of media were used in this assay: yeast nitrogen base (YNB) agar, yeast nitrogen base agar without isoleucine (YNB-iso), and yeast nitrogen base agar without tryptophan (YNB-trp). Each type of media is used to test a different mutation at a different gene locus alongside the repair mechanism induced by mutagenesis: heteroallelic *ade2* causing a change in colony colour from white to pink on non-deficient media (YNB), *trp5* mutation causing inability to grow on tryptophan-deficient media (YNB-trp), and *ilv1* mutation causing inability to grow on isoleucine-deficient media (YNB-iso) (Marshall 2007, Zimmerman 1975).

Yeast cells were incubated in yeast nitrogen base broth at 28 degrees Celsius in a shaking incubator for 18 hours prior to exposure to the air particulate matter samples. Cells were exposed to the air particulate matter samples for 2

hours at 28 degrees Celsius, and sample was subsequently washed off. Cells were plated using the drop plate method optimized to yield countable colonies within a 48-hour growth period at 28 degrees Celsius. Controls of ethidium bromide (positive) at 10 µg/mL and sterile PBS (negative) were run together with the samples. All the assays were performed in triplicate.

Data Analysis

The Ames results and mutagenicity testing with the D7 strain of *S. cerevisae* results were run separately. With the Ames test plates, mutagenicity ratios were calculated by dividing each sample revertant colony count by the number of revertant colonies of the negative control. A mutagenicity ratio greater than or equal to 2.00 is a positive result according to the two-fold rule (Cariello & Piegorsch 1996, Mortelmans & Zieger 2000). This result implies that the spontaneous reversion rate has to at least be doubled by a substance in order to be considered mutagenic. Statistically significant differences between naturally and mechanically ventilated wards, as well as the bulk, aqueous, and organic fractions of the air particulate matter were calculated through a Two-Way Analysis of Variance (ANOVA) with a Tukey's post-hoc test using the means of the absolute colony counts (ACCs) at a significance level of $p < 0.05$.

For the mutagenicity testing with the D7 strain of *S. cerevisae*, absolute colony counts (ACCs) on the YNB-iso and YNB-trp plates were averaged, and compared using a Two-Way Analysis of Variance (ANOVA) with a Tukey's post hoc test. Substances were considered mutagenic if they were found to have significantly more colonies than the negative control at significance level of $p < 0.05$. Only single colonies were grown on the YNB media plates. Samples were considered mutagenic if the color of the single colony was pink instead of its typical white appearance, and were thus, qualitatively evaluated.

RESULTS

Ames Test

All the concentrations of bulk, aqueous, and organic fractions of air particulate matter collected from naturally and mechanically ventilated wards in a public urban hospital are mutagenic following the two-fold rule (Table 1). Within naturally ventilated wards, the aqueous fraction ACCs were significantly higher than the bulk and organic fractions ($p < 0.05$) (Fig. 1). Bulk fraction ACCs were the highest in mechanically ventilated wards, but this difference was not statistically significant ($p > 0.05$) (Fig. 2).

Mutagenicity Testing Using the D7 Strain of S. cerevisae

The D7 strain of *Saccharomyces cerevisae* can test for three different mutations: mitotic crossing over with recombinational repair, mitotic gene conversion with mismatch repair, and reverse point mutation (Marshall 2007). Mitotic crossing over with recombinational repair was not detected on

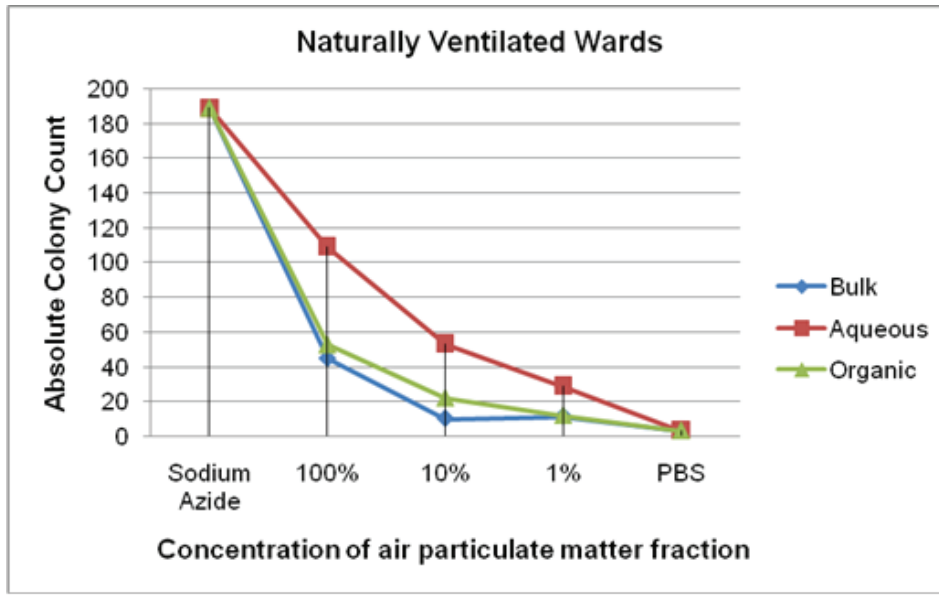

Fig. 1: Comparison of bulk, aqueous and organic fractions of air particulate matter absolute colony counts of *S typhimurium* TA 100 in naturally ventilated hospital wards.

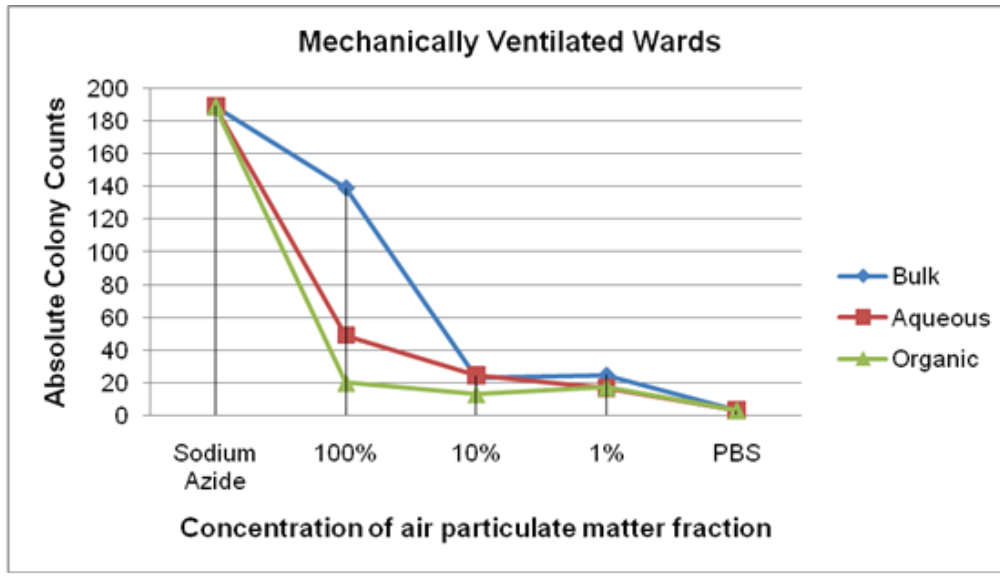

Fig. 2: Comparison of bulk, aqueous and organic fractions of air particulate matter absolute colony counts of *S typhimurium* TA 100 in mechanically ventilated hospital wards.

the YNB media (non-deficient media), as all of the colonies that grew were white in colour (Table 2).

Growth of yeast cell colonies was shown on both the YNB-trp and YNB-iso plates. Statistical significance testing of the absolute colony counts (ACCs) showed evidence of mitotic gene conversion with mismatch repair in that YNB-trp plates (tryptophan-deficient media) had significantly higher ACCs with all concentrations of the bulk, aqueous, and organic fractions of air particulate matter when compared against the negative control ($p < 0.05$) (Table 3). Reverse point mutation was also detected, as isoleucine-deficient media (YNB-iso) also showed statistically significant growth of colonies at all concentrations of all fractions ($p < 0.05$) (Table 4).

No significant differences were detected between naturally and mechanically ventilated wards ($p > 0.05$). For both the ventilation systems, the organic fraction produced significantly higher ACCs in both YNB-iso and YNB-trp media ($p < 0.05$) than bulk and aqueous fractions, especially at maximum, undiluted concentration (100% conc.) where it surpassed the positive control. Bulk and aqueous fractions were similar to one another ($p > 0.05$) with both fractions showing no significant differences among their various concentrations.

Table 1: Mutagenicity ratios (MR) of bulk, aqueous and organic fractions of air particulate matter from naturally and mechanically ventilated hospital wards with strain TA100 of *S. typhimurium.*

	Bulk				Aqueous				Organic			
	Natural	Result*	Mechanical	Result *	Natural	Result*	Mechanical	Result *	Natural	Result*	Mechanical	Result *
Sodium azide	56.78	+	56.78	+	56.78	+	56.78	+	56.78	+	56.78	+
100% conc.	13.46	+	41.79	+	32.78	+	14.77	+	15.86	+	6.06	+
10% conc.	3.10	+	7.01	+	16.02	+	7.41	+	6.66	+	5.20	+
1% conc.	3.45	+	7.46	+	8.71	+	5.16	+	3.65	+	3.95	+
PBS	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-	1.00	-

*Positive result according to the 2-point rule (Cariello & Piegorsch 1996; Mortelmans & Zieger 2000)

Table 2: Presence of pink D7 *S. cerevisae* colonies on YNB (non-deficient media) plates.

	Bulk	Aqueous	Organic
	Result*	Result*	Result*
Ethidium bromide	+	+	+
100% conc.	-	-	-
10% conc.	-	-	-
1% conc.	-	-	-
PBS	-	-	-

*Positive result indicates pink color of single colony, while negative result indicates white

Table 3: Absolute colony count for YNB-trp plates.

	Bulk				Aqueous				Organic			
	Natural	Result*	Mechanical	Result *	Natural	Result*	Mechanical	Result*	Natural	Result*	Mechanical	Result*
Ethidium bromide	6	+	6	+	6	+	6	+	6	+	6	+
100% conc.	3.67	+	2.83	+	7	+	4	+	12.65	+	7.67	+
10% conc.	8.5	+	3.17	+	8.83	+	4	+	6.83	+	3	+
1% conc.	9	+	1	+	6.5	+	3.67	+	5.17	+	4	+
PBS	0.33	-	0.33	-	0.33	-	0.33	-	0.33	-	0.33	-

*Positive result indicates statistically significant difference from the negative control (PBS) at $p < 0.05$

DISCUSSION

Air particulate matter was collected from naturally and mechanically ventilated hospital wards and fractionated into bulk, aqueous, and organic partitions. Two different methods were used to test the mutagenicity of the air particulate matter fractions: the Ames test and mutagenicity testing with the D7 strain of *S. cerevisae*.

When subject to the Ames test, all fractions at all the concentrations were found to be mutagenic, yielding a broad range of mutagenicity ratios (3.45-41.79). Mutagenic activity was shown to be dose-related with higher concentrations of air particulate matter fractions alongside higher absolute colony counts, resulting in higher mutagenicity ratios. These results suggest that the air particulate matter collected from these hospital wards are able to increase the spontaneous reversion rate of the TA 100 strain of *Salmonella typhimurium*, and thus, have mutagenic activity.

Variations in absolute colony counts (ACCs) among the different fractions hinted towards a more dominant aqueous component in naturally ventilated wards. Higher levels of non-organic indoor air pollutants such as soluble salts, heavy metals, and bioaerosols (air particulate matter from living organisms such as bacterial endotoxins, fungal spores, and animal dander) are associated with more densely populated and naturally ventilated wards (El-Sharkawy & Noweir 2014). Thus, this more dominant aqueous phase may be related to how the naturally ventilated wards of this hospital are more densely populated and more exposed to biological organisms through windows open to the outside of the hospital.

With the mechanically ventilated wards, there was a more balanced combination of aqueous and organic components. Mechanical ventilation systems such as air conditioners can contribute to both emission and dilution/dispersion of potentially harmful chemicals and bioaerosols. Poorly cleaned air conditioning units tend to become a repository for dust and animal dander, and even breeding grounds for potentially harmful and infective fungi and bacteria (Apte & Salvi 2016). Chemical coolants used to control the temperature in rooms typically come in the form of organic chemicals, sometimes chlorofluorocarbons (CFCs) that are known to be mutagenic in particular doses (Benigni et al. 2009). Air conditioners and temperature control devices have also been found to emit certain levels of volatile organic compounds, again, known mutagens that may attach themselves to air particulate matter. Mechanical ventilation systems that are poorly maintained or make use of chemicals that can be mutagenic or toxic to humans thus contribute to and circulate these indoor air pollutants. On

Table 4: Number of revertant colonies for YNB-iso plates.

	Bulk				Aqueous				Organic			
	Natural	Result*	Mechanical	Result *	Natural	Result*	Mechanical	Result *	Natural	Result*	Mechanical	Result *
Ethidium bromide	11.67	+	11.67	+	11.67	+	11.67	+	11.67	+	11.67	+
100% conc.	4.17	+	5.83	+	4.67	+	9	+	26.33	+	17.67	+
10% conc.	4.83	+	9.83	+	9.33	+	10	+	5.33	+	9.33	+
1% conc.	7.83	+	13.83	+	5.67	+	7.83	+	5.17	+	4	+
PBS	0.33	-	0.33	-	0.33	-	0.33	-	0.33	-	0.33	-

*Positive result indicates statistically significant difference from the negative control (PBS) at $p < 0.05$

the other hand, a filtration system within the mechanical ventilation system can contribute to removing any hazardous components from the circulation and air mechanical ventilation set-ups that do not make use of harmful organic chemicals and thus, would not introduce these into the air circulation of the hospital are currently available market options (Hodgson et al. 1994, Xu et al. 2015). The mechanical ventilation systems of the hospital currently have no filtration mechanisms, and there is the potential that they are contributing more to the indoor air pollution than working towards its dilution or dispersion.

While the nature of the air particulate matter with mutagenic activity in the differentially ventilated wards is hinted at by the Ames test, the manner in which it may be received and processed by the human body is reflected in the mutagenicity testing with the D7 strain of *S. cerevisae*. This is because this model is eukaryotic system with the natural capacity for metabolizing substances that are taken in and intact DNA repair mechanisms for when damage is incurred. Results showed that regardless of ventilation system, the organic fraction produced the greatest mutagenic activity with absolute colony counts (ACCs) greater than even the positive control for both types of media, representing mitotic gene conversion (YNB-trp) and reverse point mutation (YNB-iso). Mitotic crossing over, on the other hand, was not detected on the YNB media. Polycyclic aromatic hydrocarbons (PAHs) tend to display levels of toxicity prior to break down, but their toxic and mutagenic effects, and thus, their harmfulness can increase following metabolism into their simpler chemical constituents (Balbo et al. 2014). Previous work has shown that even at low levels, these volatile and semi-volatile organic compounds can display notable levels of toxicity and mutagenicity (Hodgson et al. 1994).

The results of both the assays support one another in that the sampled air particulate matter is mutagenic. The YNB-iso plates test for a reverse point mutation to the *ilv1* locus on *Saccharomyces cerevisae*, that corrects the induced isoleucine auxotrophy on the strain similar to how the TA 100 strain is designed to test for the back-mutation of the induced histidine auxotrophy at the *hisG46* locus of *Salmonella typhimurium*. Positive results in both assays at all concentrations of all fractions support the presence of an increased rate of reverse point mutations in the presence of the air particulate matter fractions.

CONCLUSION AND FUTURE DIRECTIONS

In summary, the air particulate matter in the public urban hospital sampled has mutagenic activity according to both the Ames test and mutagenicity testing with the D7 strain of *S. cerevisae*. Although the nature of the dominant components of the mutagen may vary according to the ventilation system applied to the ward, it appears that intake and processing of the mutagen in eukaryotic systems is fairly constant across ventilation type. This may then suggest that policies and practices to improve the air quality of the hospital should be applied to wards of both types of ventilation systems, and move towards minimizing the concentrations of organic pollutants.

It must be noted that these significant results were detected using samples that concentrated 3 days'-worth of air particulate matter into small volume containers. This may not necessarily reflect the air being breathed in larger volume hospital wards, and thus, the study should cause any kind of panic about the harmfulness of the air in the hospital. In-depth fractionation and analysis of the different of the components of the indoor air, as well as monitoring the quality of the air alongside relevant practices and policies geared towards improving the air quality would lead to a more complete understanding, and thus, a more detailed action plan for improving hospital air quality at the administrative level.

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