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EDITORIAL BOARD

SCHOOL OF ARTS AND SCIENCES

Young Adults and the Consumption of Electronic Nicotine-Based Products

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Abstract

The young adult population is exposed to varieties of tobacco products. This can cause young adults to feel inclined toward using these products. The reason why young adults are trying or using electronic nicotine-based products comes from the variety of flavors offered, the idea that it is better than cigarettes, curiosity, pressure from peers, or to quit/reduce their tobacco use (Luzius et al., 2022). This study examined the relationship between the young adult population and the consumption of electronic nicotine-based products. This study used a non-probability availability sample which was distributed to 85 Cabrini University students in the fall semester of 2022. The survey was used to examine if electronic nicotine-based tobacco products were utilized by the students. Hypothesized in this study was that young adults are more likely to use electronic nicotine-based products if they are exposed to social media advertisements and posts and also if they observe their peers using these products. It was also hypothesized that masculinity will cause males to be more likely to consume electronic nicotine-based products. Results showed that peer pressure does influence the young adult population when it comes to using these tobacco products. Young adults who live with someone who does not use any form of tobacco product were found to be more likely to not use electronic nicotine-based products.

Introduction

According to the CDC, nearly 9 out of 10 adults who smoke or use electronic nicotine-based smoking products daily first tried smoking at the age of 18 years old, with 99% of these people first tried smoking by the age of 26 years old. In the United States, each day more than 1,600 youths will try their first electronic nicotine-based smoking product with 200 of them continuing to use these types of products daily (CDC, 2022). A majority of the people who are

using electronic nicotine-based smoking happen to be in the young adult age category, 18 years old to 25 years old (Luziuz, Dobbs, & Jozkowski, 2020). Young adults are now consuming and using more electronic nicotine-based smoking products, which is a shift from young adults historically smoking primarily cigarettes. This study explored how the young adult population is being shaped and consuming electronic nicotine-based smoking products.

Many studies have been completed on smoking habits among teenagers and young adults from years prior. In today's society, smoking habits and forms of smoking have changed and are not the same as they once were. Young adults are now consuming and using more electronic nicotine-based smoking products. Many of the popular electronic smoking products among the young adult age group are smoking e-cigarettes, vaping, and Juul. Other than curiosity, "the second most frequently cited reason for first using a Juul, use by friends, is also consistent with previous literature on e-cigarettes and other tobacco products implying the importance of social influence" (Ickes et al., 2020). This study focused on how social media influence, peer pressure, and masculinity affect young adults when it comes to electronic nicotine-based smoking products.

Literature Review

The literature on smoking and the use of electronic nicotine-based smoking products focused on three major themes: how peer pressure affects the use of these types of products among young adults (Luzius et al., 2020; Dobbs, Clawson, Gowin, & Cheney, 2020); how social media has influenced the use of these products (Luzius et al., 2020; Sawdey, Hancock, Messner, & Prom-Wormley, 2017; Ickes et al., 2020) and how the male gender is effected by electronic nicotine-based smoking products (Sawdey et al., 2017; Luzius et al., 2020; Clendennen et al.,

2019; Ickes et al., 2020; Dobbs et. al., 2020). Each of these themes is important for understanding why the young adult population uses electronic nicotine-based smoking products.

Peer Pressure

The first theme in this literature on electronic nicotine-based smoking products and young adults explored the relationship between the use of these products and how peers affect the type of products being consumed. In the research completed by Luzius et al (2020), it was found that out of the 157 people who participated in the survey 74 participants tried smoking electronic nicotine-based smoking products because of peer pressure as well as 25 participants tried one because of recreation (because it seemed cool). This research also concluded that college campuses have one of the highest concentrated peer pressure influences of all time. This is because many of these students live in an environment with people ranging from the ages of as young as 18 years old (sometimes 17 years old) to 25 years old. These college students are also living in an environment on their own where they do not have parental guidance in their everyday lives. Their main source of guidance is their peers and the influence they have on each other (Luzius et al., 2020).

One of the researches completed on peer pressure and the use of electronic nicotine-based smoking products found that more than half of the college students have seen their peers post and advertise via social media the use of e-cigarettes and other vaping products (Sawdey et al., 2017). Viewing their peers post images online of them using and consuming a product can make a person feel the need to do the same thing. It was found that peer pressure causes these young adults to feel obligated to use the products they see their acquaintances use in hopes of fitting in, looking cool, and being able to participate in conversations (Luzius et al., 2020). Many also choose to use electronic nicotine-based smoking products out of curiosity from seeing their peers

consume them (Sawdey et al., 2017; Luzius et al. 2020). People in the young adult category value the respect of their peers and want their approval. They feel that smoking these products can help ease the approval and help make them seem cooler to other peers.

Social Media Influence

The second theme in this literature on electronic nicotine-based smoking products and young adults explored the relationship between the use of these products and how social media influences these types of products being consumed. From the research conducted by Sawdey, it was found that social media is encouraging young adults to use e-cigarettes. It was found that social media is becoming a platform for providing the "indirect approach to encourage the use of e-cigarettes and a direct marketing approach for an e-cigarette to push onto potential users" (Sawdey et al., 2017). Over half of the participants in Sawdey's research claimed they had access to Facebook, Snapchat, Instagram, or Twitter. Within this group of participants, every one of them claimed they either saw a direct ad post from an electronic nicotine-based smoking product or saw their peers post using the said product. This caused many to feel an obligation to either consume this product or feel ashamed for not partaking in consuming the product (Sawdey et al., 2017).

In the research conducted by Ickes et al (2020), it was found that there was one brand, Juul, that was purposely marketing to the young adult age category via the media. Through his extensive research of the young adult population, he was able to inform them of the conclusions that he made. It was concluded in his study that he was "able to open the minds of the participants in the Juul use research to show them how the media has influenced them to use their products" (Ickes et al., 2020). Icke's research was also able to have an understanding of why this age group used Juul products instead of others, "it was able to demonstrate that young

adults are choosing Juul products because they are being targeted by these certain companies" (Ikes et al. 2020).

Gender and Race

The third theme in the literature on electronic nicotine-based smoking products and young adults explored the relationship between the use of these products and how all genders and races are affected by the products. The results of the multiple research had different views on which races were using these products more than others depending on where the research was being conducted. The research conducted by (Ickes et al., 2020; Luzius et al. 2020; Sawdey et al. 2017) found that the majority of the respondents to the research were female. It was found in all three of these research that more than 67% of the respondents were females. Males were less likely to respond to the survey, which is why we see an increase in female participation in the research.

Almost all races were represented in the research when it came to electronic nicotine-based smoking products and young adults. We see a majority of the participants in the research being White non-Hispanic, with Hispanic and/or Black is either second or third (depending on where the research was conducted) and Asian being represented the least (Sawdey et al., 2017; Luzius et al., 2020; Clendennen et al., 2019; Ickes et al., 2020; Dobbs et. al., 2020). In one study that was conducted, it was found that "the results [also] showed that the participants were mainly White (81.8%) and were mainly females (61.8%). There were 77.2% of participants in this research who had smoked a regular cigarette in their lifetime and 25.6% of the participants had smoked a regular cigarette in the past 30 days" (Luzius et al., 2020). From this research, we can see that a common theme among participants and those who completed the research were mainly White and females.

The Present Study on Electronic Nicotine-Based Smoking Products

Young adults from ages 18 to 25 years old were the focus of the study on electronic nicotine-based smoking products. The young adults found in the study who use these products ranged from all different backgrounds; White, Black, Hispanic, Asian, upper class, middle class, lower class, etc. To fully explore the reasons for these young adults using electronic nicotine-based smoking products, it is important to understand what type of people this age group looks up to; i.e., celebrities, friends, and family.

The following questions emerge from this literature: How does social media influence these young adults to use products of vaping, e-cigarettes, and electronic nicotine smoking? How does the use of these products at a young age affect the health and well-being of these young adults? This study extends the literature on young adults' use of electronic nicotine-based products. It also extends the literature on the peer pressure these young adults feel to use these products by focusing on the peer groups around those who smoke.

Hypotheses

i. If young adults are exposed to vape products from social media advertisements and posts, then they are more likely to use said products.
ii. Young adults who observe their peers using vape products are more likely to use said products.
iii. Young adult males will be the most common gender using vaping products due to masculinity having a connection to vaping rates.

Methodology

Sample and procedure

For this study, the data that was collected consisted of a nonprobability, convenience sample. Data was collected from N=85 participants from Cabrini University. In the fall semester of 2022, electronic surveys were distributed to Cabrini University students in different classes and majors as well as athletes on different sports teams at the university. Electronic surveys were created using Google Forms and were sent out via email. No paper surveys were used. The surveys that were presented to the students were completely voluntary. The research study is designed to measure young adults and their consumption of electronic nicotine-based products.

Demographics

The sample consisted of 71.8% (n=61) female respondents, 23.5% (n=20) male respondents, and 2.4% (n=2) others. From the students who completed the survey 20% (n=17) said they were 18 years old, 27.1% (n=23) were 19 years old, 11.8% (n=10) were 20 years old, 17.6% (n=15) were 21 years old, 10.6% (n=9) were 22 years old, and 8.2% (n=7) were 23 years old or older. There were 87.1% (n=74) who identified as not being Hispanic, 7.1% (n=6) who identified as Mexican, Mexican American, Chicano, or Chicana, 3.5% (n=3) who identified as Puerto Rican, and 1.2% (n=1) who identified as Another Hispanic, Latino, Latina, or Spanish origin. The racial distribution of the sample was composed of 64.7% (n=55) White, 22.4% (n=19) Black, 3.5% (n=3) Asian, and 8.2% (n=7) who identified as other. See table 1 for the sample demographic breakdown.

Table 1

Sample Demographics

	Frequency	***Valid Percent***
Gender		
Female	61	71.8
Male	20	23.5
Other	2	2.4
Age		
18	17	20.0
19	23	27.1
20	10	11.8
21	15	17.6
22	9	10.6
23 and older	7	10.5
Hispanic, yes or no		
No, not of Hispanic origin	74	87.1
Yes, Mexican, Mexican American, Chicano, or Chicana	6	7.1
Yes, Puerto Rican	3	3.5
Yes, Another Hispanic, Latino, Latina, or Spanish origin	1	1.2
Race		
White	55	64.7
Black	19	22.4
Asian	3	3.5
Other	7	8.2

Variables of interest

The independent variable for this study is exposure to electronic nicotine-based products. Exposure to the said product can be defined as "exposure to tobacco marketing and perception of peer tobacco use" (Kreitzberg et al., 2018). This independent variable was able to be measured by asking the participants two questions. The first question: "During the past 30 days, did you

receive advertisements from a tobacco company through:" (1= I did not receive advertisements from a tobacco company during the past 30 days, 2= The mail, 3= E-mail, 4= Social media (Twitter, Facebook, Tik Tok, Snapchat, Instagram, etc.), 5= A text message, 6= Some other source not listed here, 7= Multiple sources)(YTS, 2018/19). 62.4% (n=53) of the participants had never received advertisements from a tobacco company during the past 30 days. The second question: "When you are using the internet, how often do you see advertisements or promotions for e-cigarettes?" (1= Never, 2= Rarely, 3= Sometimes, 4= Most of the time, 5= Always, 6= I do not use the internet) (YTS, 2018/19). 35.3% (n=30) of the participants in the survey had responded sometimes. See table 2 for the independent variable breakdown.

The second independent variable for this study was the observation of the consumption of electronic nicotine-based products. Observation of the said products can be defined as feeling the influence to use e-cigarettes and other nicotine-based products due to observing peers consuming them (Wong et al., 2019). This independent variable was able to be measured by asking the participants two questions. The first question: "Have you ever seen anyone using an e-cigarette, such as JUUL, Vuse, or Blu, in any locations in or around your school?" (1= No, 2= Yes, inside a school bathroom or locker room, 3= Yes, inside a classroom, 4= Yes, inside some other area of the school (hallway, cafeteria), 5= Yes, somewhere else not listed here, 6= Yes, outside of the school, such as in the parking lot, sidewalk, sports field, or other school grounds) (CDC, 2022). 75.3% (n=64) had responded yes they had seen someone outside of the school such as in the parking lot, sidewalk, sports field, or other school grounds using an e-cigarette product. The second question: "Does anyone who lives with you now…?" (1= Smoke cigarettes, 2= Smoke cigars, cigarillos, or little cigars, 3= Use chewing tobacco, snuff, or dip, 4= Use e-cigarettes, 5= Smoke tobacco in a hookah or waterpipe, 6= Smoke pipes filled with tobacco (not hookah or

waterpipes), 7= Use snuff, 8= Use dissolvable tobacco products, 9= Smoke bidis (small brown cigarettes wrapped in a leaf), 10= Use heated tobacco products, 11= No one who lives with me now uses any form of tobacco, 12= Other products not listed, 13= Multiple products) (CDC, 2022). 62.4% (n=53) of the participants had responded to no one who lives with them now uses any form of tobacco products. See table 2 for the independent variable breakdown.

The third independent variable for this study was gender. The question to measure gender was: "What is your gender?" (1=Male, 2=Female, 3= Other) (CDC, 2022). From the results, it was found that 23.5% of males (n=20) participated in the survey along with 71.8% of females (n=61), 2.4% (n=2) as other, and 2.4% (n=2) who did not want to disclose their gender. See table 2 for the independent variable breakdown.

Table 2

Independent Variables

	Frequency	***Valid Percent***
Past 30 days did you receive ads from tobacco company		
I did not receive ads	53	62.4
The mail	1	1.2
Social media (Twitter, Facebook, Tik Tok, Snapchat, Instagram)	22	25.9
A text message	1	1.2
Some other source not listed	2	2.4
Multiple sources	5	5.9
How often do you see ads or promotions for e-cigarettes		
Never	11	12.9
Rarely	26	30.6
Sometimes	30	35.3
Most of the time	14	16.5
Always	3	3.5

I do not use the internet	1	1.2
Seen people using e-cigarettes in/around school		
No	6	7.1
Yes, inside a school bathroom or locker room	4	4.7
Yes, inside a classroom	-	-
Yes, inside some other area of the school (hallway, cafeteria)	3	3.5
Yes, somewhere else not listed here	8	9.4
Yes, outside of the school, such as in the parking lot, sidewalk, sports field, or other school grounds	64	75.3
Does anyone who lives with you now…		
Smoke cigarettes	3	3.5
Smoke cigars, cigarillos, or little cigars	3	3.5
Use chewing tobacco, snuff, dip	2	2.4
Use e-cigarettes	13	15.3
Smoke tobacco in a hookah or waterpipe	2	2.4
Smoke pipes filled with tobacco (not hookah or waterpipes)	-	-
Use snuff	-	-
Use dissolvable tobacco products	-	-
Smoke bidis (small brown cigarettes wrapped in a leaf)	-	-
Use heated tobacco products	1	1.2
No one who lives with me now uses any form of tobacco	53	62.4

Other products not listed	6	7.1
Multiple products	2	2.4
Gender		
Male	20	23.5
Female	61	71.8
Other	2	2.4

The dependent variable for this study is the use of electronic nicotine-based products. The use of these said products can be defined as the current e-cigarette use or have used e-cigarettes in their life (Sawdey et al., 2017). Two questions were asked to measure the use of these products. Participants were first asked: "In total, on how many days have you used e-cigarettes in your entire life?" (1= 0 days, 2= 1 day, 3= 2 to 10 days, 4= 11 to 20 days, 5= 21 to 50 days, 6= 51 to 100 days, 7= over 100 days) (YTS, 2018/19). 50.6% (n=43) of participants responded to 0 days. Participants were then asked: "When was the last time you used an e-cigarette, even one of two times?" (1= Never, 2= Earlier today, 3= Not today, but sometime during the past 7 days, 4= Not during the past 7 days, but sometime during the past 30 days, 5= Not during the past 30 days, but sometime during the past 6 months, 6= Not during the past 6 months, but sometime during the past year, 7= 1 to 4 years ago, 8= 5 or more years ago)(CDC, 2022). 50.6% (n= 43) of participants responded to never. See table 3 for the dependent variable breakdown.

Table 3

Dependent Variable

	Frequency	*Valid Percent*
Total Days of using e-cigarettes		
0 Days	43	50.6
1 Day	6	7.1
2 to 10 Days	7	8.2
11 to 20 Days	7	8.2
21 to 50 Days	2	2.4
Over 100 Days	19	22.4
Last time Used an e-cigarette		
Never	43	50.6
Earlier Today	13	15.3
Not today, but sometime during the past 7 days	5	5.9
Not during the past 7 days, but sometime during the past 30 days	5	5.9
Not during the past 30 days, but sometime during the past 6 months	4	4.7
Not during the past 6 months, but sometime during the past year	3	3.5
1 to 4 years ago	11	12.9
5 or more years ago	1	1.2

Below, is the overall descriptive statistics table. Within this table, all of the variables of interest are represented. The majority of the variables had a very high response rate. See table 4 for the complete breakdown of descriptive statistics.

Table 4

Variable Descriptive Statistics

Variable	*N*	*Mean*	*Median*	*Mode*	*Range*	*Standard Deviation*
Age	81	-	3	2	7	-
Gender	83	-	2	2	2	-
Hispanic	84	-	1	1	3	-
Race	84	-	1	1	5	-
Past 30 days did you receive ads from tobacco company	84	-	1	1	6	-
How often do you see ads or promotions for e-cigarettes	85	-	3	3	5	-
Seen people using e-cigarettes in/around school	85	-	7	7	6	-
Does anyone who lives with you now…	85	-	11	11	12	-
Total days of using e-cigarette	84	-	1	1	6	-
Last time used an e-cigarette	85	-	1	1	7	-

Results

Table 5 shows the results of the chi-square crosstabulation that was conducted to analyze the relationship between the gender of the participant of the study and the total number of days of using an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was not significant, $X^2(10, N=82)=8.330, p=.597$. The correlation between the two variables, Cramer's V, is weak, at v=.225. In addition, table 6 shows the results of the chi-square crosstabulation between the two variables, the gender of the participant of the study, and the last time the participant used an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was not significant, $X^2(14, N=83)=7.357, p=.920$. The correlation between the two variables, Cramer's V, is weak, at v=.211.

Table 5

Chi-square crosstabulation of the gender of the participant and the total number of days of using an e-cigarette(s).

		Male	*Female*	*Other*	*Total*
Total Days of Using E-Cigarettes	**0 Days**	70%	43.3%	100%	51.2%
	1 Day	0%	10%	0%	7.3%
	2 to 10 Days	5%	10%	0%	8.5%
	11 to 20 Days	5%	10%	0%	8.5%
	21 to 50 Days	5%	1.7%	0%	2.4%
	Over 100 Days	15%	25%	0%	22%
	Total	100%	100%	100%	100%

Table 6

Chi-square crosstabulation of the gender of the participant and the last time they used an e-cigarette(s).

		Male	*Female*	*Other*	*Total*
Last Time Used an E-Cigarette	**Never**	65%	44.3%	100%	50.6%
	Earlier Today	5%	18%	0%	14.5%
	Not Today, But Sometime During the Past 7 Days	10%	4.9%	0%	6%

	Not During the Past 7 Days, But Sometime During the Past 30 Days	5%	6.6%	0%	6%
	Not During the Past 30 Days, But Sometime During the Past 6 Months	5%	4.9%	0%	4.8%
	Not During the Past 6 Months, But Sometime During the Past Year	0%	4.9%	0%	3.6%
	1 to 4 Years Ago	10%	14.8%	0%	13.3%
	5 or More Years Ago	0%	1.6%	0%	1.2%
	Total	100%	100%	100%	100%

Table 7 shows the results of the chi-square crosstabulation that was conducted to examine the relationship between the participants who have seen people using e-cigarettes in or around school and the total number of days of using an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was not significant, $X^2(20, N=84)=19.226, p=.507$. The correlation between the two variables, Cramer's V, is weak, at v=.239. In addition, table 8 shows the results of the chi-square crosstabulation between two variables, the relationship of the participants who have seen people using e-cigarettes in or around school, and the last time the participant had used an e-cigarette(s). From the chi-square

crosstabulation, the relation between the two variables was not significant, $X^2(28, N=85)=31.077, p=.314$. The correlation between the two variables, Cramer's V, is moderate, at v=.302. Furthermore, table 9 shows the results of the chi-square crosstabulation between two variables, the relationship of the participants who live with someone who uses some type of tobacco product, and the total number of days of using an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was approaching significance, $X^2(40, N=84)=53.921, p=.070$. The correlation between the two variables, Cramer's V, is moderate, at v=.358. Also, table 10 shows the results of the chi-square crosstabulation between two variables, the relationship of the participants who live with someone who uses some type of tobacco product, and the last time the participant had used an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was significant, $X^2(56, N=85)=102.433, p<.001$. The correlation between the two variables, Cramer's V, is moderate, at v=.415.

Table 7

Chi-square crosstabulation of the participants who have seen people using e-cigarettes in or around school and the total number of days of using an e-cigarette(s).

		0 Days	*1 Day*	*2 to 10 Days*	*11 to 20 Days*	*21 to 50 Days*	*Over 100 Days*	*Total*
Seen People Using E-Cigarettes In/Around School	**No**	14%	0%	0%	0%	0%	0%	7.1%
	Yes, Inside a School Bathroom or Locker Room	4.7%	0%	0%	0%	0%	10.5%	4.8%
	Yes, Inside Some Other Area of the School (Hallway, Cafeteria, etc.,)	4.7%	16.7%	0%	0%	0%	0%	3.6%

	Yes, Somewhere Else Not Listed Here	9.3%	33.3%	0%	14.3%	0%	5.3%	9.5%
	Yes, Outside of the school such as in the parking lot, sidewalk, sports field	67.4%	50%	100%	85.7%	100%	84.2%	75%
	Total	100%	100%	100%	100%	100%	100%	100%

Table 8

Chi-square crosstabulation of the participants who have seen people using e-cigarettes in or around school and the last time they used an e-cigarette(s).

		Never	***Earlier Today***	***Not Today, But Sometime During the Past 7 Days***	***Not During the Past 7 Days, But Sometime During the Past 30 Days***	***Not During the Past 30 Days, But Sometime During the Past 6 Months***	***Not During the Past 6 Months, But Sometime During the Past Year***	***1 to 4 Years Ago***	***5 or More Years Ago***	***Total***
Seen People Using E-Cigarettes In/Around School	**No**	14%	0%	0%	0%	0%	0%	0%	0%	7.1%
	Yes, Inside a School Bathroom or Locker Room	4.7%	15.4%	0%	0%	0%	0%	0%	0%	4.7%
	Yes, Inside Some Other Area of the School (Hallway, Cafeteria, etc.,)	4.7%	0%	0%	0%	0%	0%	9.1%	0%	3.5%
	Yes, Somewhere Else Not Listed Here	9.3%	0%	0%	60%	0%	0%	9.1%	0%	9.4%
	Yes, Outside of the school such as in the parking lot, sidewalk, sports field	67.4%	84.6%	100%	40%	100%	100%	81.8%	0%	75.3%
	Total	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table 9

Chi-square crosstabulation of the participants who live with someone who uses some type of tobacco product and the total number of days of using an e-cigarette(s).

		0 Days	*1 Day*	*2 to 10 Days*	*11 to 20 Days*	*21 to 50 Days*	*Over 100 Days*	*Total*
Does Anyone Who Lives With You Now…	**Smoke Cigarettes**	4.7%	0%	0%	0%	0%	5.3%	3.6%
	Smoke Cigars, Cigarillos, Or Little Cigars	2.3%	0%	14.3%	14.3%	0%	0%	3.6%
	Use Chewing Tobacco, Snuff, or Dip	2.3%	0%	0%	14.3%	0%	0%	2.4%
	Use E-Cigarettes	2.3%	0%	28.6%	0%	50%	47.4%	15.5%
	Smoke Tobacco in a Hookah or Waterpipe	4.7%	0%	0%	0%	0%	0%	2.4%
	Use Heated Tobacco Products	0%	0%	0%	0%	0%	5.3%	1.2%
	No One Who Lives With Me Now Uses Any Form of Tobacco	74.4%	100%	57.1%	57.1%	50%	26.3%	61.9%
	Other Products Not Listed	9.3%	0%	0%	14.3%	0%	5.3%	7.1%
	Multiple Products	0%	0%	0%	0%	0%	10.5%	2.4%
	Total	100%	100%	100%	100%	100%	100%	100%

Table 10

Chi-square crosstabulation of the participants who live with someone who uses some type of tobacco product and the last time they used an e-cigarette(s).

		Never	*Earlier Today*	*Not Today, But Sometime During the Past 7 Days*	*Not During the Past 7 Days, But Sometime During the Past 30 Days*	*Not During the Past 30 Days, But Sometime During the Past 6 Months*	*Not During the Past 6 Months, But Sometime During the Past Year*	*1 to 4 Years Ago*	*5 or More Years Ago*	*Total*
Does Anyone Who Lives With You Now…	**Smoke Cigarettes**	4.7%	0%	0%	0%	0%	0%	9.1%	0%	3.5%
	Smoke Cigars, Cigarillos, Or Little Cigars	2.3%	0%	0%	20%	0%	0%	9.1%	0%	3.5%
	Use Chewing Tobacco, Snuff, or Dip	2.3%	0%	0%	0%	0%	0%	9.1%	0%	2.4%
	Use E-Cigarettes	2.3%	61.5%	20%	20%	50%	0%	0%	0%	15.3%
	Smoke Tobacco in a Hookah or Waterpipe	4.7%	0%	0%	0%	0%	0%	0%	0%	2.4%
	Use Heated Tobacco Products	0%	0%	0%	0%	0%	33.3%	0%	0%	1.2%
	No One Who Lives With Me Now Uses Any Form of Tobacco	76.7%	30.8%	40%	60%	50%	33.3%	72.7%	0%	62.4%
	Other Products Not Listed	7%	0%	20%	0%	0%	33.3%	0%	100%	7.1%
	Multiple Products	0%	7.7%	20%	0%	0%	0%	0%	0%	2.4%
	Total	100%	100%	100%	100%	100%	100%	100%	100%	100%

Below, table 11 shows the results of the chi-square crosstabulation that was conducted for the exploration of the relationship between the participants who had often received advertisements or promotions for e-cigarettes and the total number of days of using an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was not significant, $X^2(25, N=84)=26.894, p=.361$. The correlation between the two variables, Cramer's V, is weak, at v=.253. In addition, table 12 shows the results of the chi-square crosstabulation that was conducted to inspect the relationship between the participants who had often received advertisements or promotions for e-cigarettes and the last time the participants had used an e-cigarette(s). The correlation between the two variables, Cramer's V, is weak, at v=.289. Furthermore, table 13 shows the results of the chi-square crosstabulation that was conducted to analyze the relationship between the participants who had received advertisements from tobacco companies in the past 30 days and the total number of days of using e-cigarettes (s). From the chi-square crosstabulation, the relation between the two variables was not significant, $X^2(25, N=83)=15.099, p=.939$. The correlation between the two variables, Cramer's V is weak, at v=.191. Lastly, table 14 shows the results of the chi-square crosstabulation that was conducted to examine the relationship between the participants who had received advertisements from tobacco companies in the past 30 days and the last time the participants had used an e-cigarette(s). From the chi-square crosstabulation, the relation between the two variables was not significant, $X^2(35, N=84)=29.612, p=.726$. The correlation between the two variables, Cramer's V, is weak, at v=.266.

Table 11

Chi-square crosstabulation of the participants who had often received advertisements or promotions for e-cigarettes and the total number of days of using an e-cigarette(s).

		0 Days	*1 Day*	*2 to 10 Days*	*11 to 20 Days*	*21 to 50 Days*	*Over 100 Days*	*Total*
How Often Do You See Ads or Promotions for E-Cigarettes	**Never**	11.6%	0%	14.3%	14.3%	0%	21.1%	13.1%
	Rarely	25.6%	33.3%	28.6%	14.3%	50%	47.4%	31%
	Sometimes	44.2%	33.3%	57.1%	42.9%	0%	10.5%	35.7%
	Most of the Time	18.6%	33.3%	0%	28.6%	50%	5.3%	16.7%
	Always	0%	0%	0%	0%	0%	10.5%	2.4%
	I Do Not Use the Internet	0%	0%	0%	0%	0%	5.3%	1.2%
	Total	100%	100%	100%	100%	100%	100%	100%

Table 12

Chi-square crosstabulation of the participants who had often received advertisements or promotions for e-cigarettes and the last time the participants had used an e-cigarette(s).

		Never	*Earlier Today*	*Not Today, But Sometime During the Past 7 Days*	*Not During the Past 7 Days, But Sometime During the Past 30 Days*	*Not During the Past 30 Days, But Sometime During the Past 6 Months*	*Not During the Past 6 Months, But Sometime During the Past Year*	*1 to 4 Years Ago*	*5 or More Years Ago*	*Total*
How Often Do You See Ads or Promotions for E-Cigarettes	**Never**	9.3%	15.4%	40%	0%	0%	0%	27.3%	0%	12.9%
	Rarely	25.6%	46.2%	0%	60%	50%	66.7%	18.2%	0%	30.6%
	Sometimes	44.2%	15.4%	20%	40%	50%	33.3%	18.2%	100%	35.3%
	Most of the Time	18.6%	7.7%	20%	0%	0%	0%	36.4%	0%	16.5%
	Always	2.3%	7.7%	20%	0%	0%	0%	0%	0%	3.5%
	I Do Not Use the Internet	0%	7.7%	0%	0%	0%	0%	0%	0%	1.2%
	Total	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table 13

Chi-square crosstabulation of the participants who had received advertisements from tobacco companies in the past 30 days and the total number of days of using an e-cigarette(s).

		0 Days	*1 Day*	*2 to 10 Days*	*11 to 20 Days*	*21 to 50 Days*	*Over 100 Days*	*Total*
Past 30 Days Did You Receive Ads From Tobacco Companies	**I Did Not Receive Ads From a Tobacco Company During the Past 30 Days**	64.3%	50%	85.7%	57.1%	50%	63.2%	63.9%
	The Mail	2.4%	0%	0%	0%	0%	0%	1.2%
	Social Media (Twitter, Facebook, Tik Tok, Snapchat, Instagram)	26.2%	50%	14.3%	28.6%	50%	21.1%	26.5%
	A text message	0%	0%	0%	0%	0%	5.3%	1.2%
	Some Other Source Not Listed Here	0%	0%	0%	14.3%	0%	5.3%	2.4%
	Multiple Sources	7.1%	0%	0%	0%	0%	5.3%	4.8%
	Total	100%	100%	100%	100%	100%	100%	100%

Table 14

Chi-square crosstabulation of the participants who had received advertisements from tobacco companies in the past 30 days and the last time the participants had used an e-cigarette(s).

		Never	*Earlier Today*	*Not Today, But Some time During the Past 7 Days*	*Not During the Past 7 Days, But Sometime During the Past 30 Days*	*Not During the Past 30 Days, But Sometime During the Past 6 Months*	*Not During the Past 6 Months, But Sometime During the Past Year*	*1 to 4 Years Ago*	*5 or More Years Ago*	*Total*
Past 30 Days Did You Receive Ads From Tobacco Companies	**I Did Not Receive Ads From a Tobacco Company During the Past 30 Days**	61.9%	61.5%	40%	80%	75%	66.7%	63.6%	100%	63.1%
	The Mail	2.4%	0%	0%	0%	0%	0%	0%	0%	1.2%

	Social Media (Twitter, Facebook, Tik Tok, Snapchat, Instagram)	26.2%	30.8%	40%	20%	25%	0%	27.3%	0%	26.2%
	A text message	0%	7.7%	0%	0%	0%	0%	0%	0%	1.2%
	Some Other Source Not Listed Here	0%	0%	0%	0%	0%	33.3%	9.1%	0%	2.4%
	Multiple Sources	9.5%	0%	20%	0%	0%	0%	0%	0%	6%
	Total	100%	100%	100%	100%	100%	100%	100%	100%	100%

Discussion

The purpose of this study was to examine whether young adults are partaking in the consumption of electronic nicotine-based products. From previous studies that had been conducted on this topic, the hypothesis comprised that exposure to tobacco products advertised and posted on social media will cause the young adult to consume the product. The results found in the chi-square crosstabulation, *table 14,* did not support the hypothesis. It was found that 26.2% (n=11) of the participants who never smoked an e-cigarette had received an advertisement from tobacco companies through social media. It was also found in the chi-square crosstabulation, *table 13,* that 64.3% (n=27) of the participants did not receive an advertisement from a tobacco company during the past 30 days and had used an e-cigarette for a total of 0 days. In previous literature, it was found that social media platforms are an indirect source of causing young adults to use tobacco products. When young adults view the media and see posts and advertisements on these said products it tends to give them the curiosity to try e-cigarettes. In

one previous study, which had a sample size of 258 college students, it was found that over 69% of the participants had reported lifetime e-cigarette use, current e-cigarette use, and dual users of both e-cigarettes and cigarettes (Sawdey et. al., 2017). This study results found that students at Cabrini University are not influenced by social media and consume electronic-nicotine-based products.

The second hypothesis established that young adults who observe their peers using electronic nicotine-based products are more likely to use the products. The results found in the chi-square crosstabulation, *table 10,* did support the hypothesis. It was found that 76.7% (n=33) of the participants who live with someone who does not use any form of tobacco product reported never the last time they used an e-cigarette. The hypothesis was also found to be supported in the chi-square crosstabulation, *table 9,* which examined the relationship between the participants who live in adults with someone who uses some type of tobacco product and the total number of days of using e-cigarettes. The results showed that 74.4% (n=32) of the participants reported having used an e-cigarette for a total of 0 days and living with people who do not use any form of tobacco products. Previous literature had found that peer pressure is a critical element in young adults' early experimentation with tobacco products. When young adults are exposed to their peers, whether it be their family, friends, classmates at school, or people in their community, they are more likely to have a curiosity about trying these products (Luzius et al., 2020). This study's results found that peer pressure does have an effect on the students at Cabrini University. If the students' peers are not using tobacco products, they are more likely to not use them.

Lastly, the third hypothesis was formed that males will be the most common gender to use electronic nicotine-based products since masculinity correlates with tobacco use

rates. The results found in the chi-square crosstabulation, *table 5,* did not support the hypothesis. It was found that 70% (n=14) of males reported having used an e-cigarette for a total of 0 days whereas 43.3% (n=26) of females reported the same thing. The results of the chi-square crosstabulation, *table 6,* also did not support the hypothesis that males will be more likely than females to use e-cigarettes. It was found that 65% (n=13) of the males report never the last time they had used an e-cigarette whereas 44.3% (n=27) of females reported the same thing. In previous literature, it was found that males use tobacco products at higher rates than females. The masculinity of males smoking came from the idea that males could appear to be dominant over females as well as have control (Ickes et al., 2020). This study results found that students at Cabrini University are not influenced by the aspects of male masculinity and consuming electronic-nicotine-based products.

When it comes to research studies, there is some form of limitations that seek to exist. One of the limitations evident when completing this study was the *sensitive topic.* Having a sensitive topic researched was difficult since it could cause people to be angry or upset when completing the survey. This could also cause people to feel distressed when completing the survey questions since it is considered an illegal activity or ethically questionable behavior to those who are not of age and are partaking in the use of e-cigarette products. Another limitation that occurred during the study was the *sample size*. The sample size of this study included 85 participants, 61 who identified as females, 20 who identified as males, 2 who identified as other, and 2 who didn't disclose their gender. There was a disproportion when it came to the male and female response rates. Another limitation that occurred was *time constraints*. The study had to be conducted in a short period, which didn't allow there to be any follow-up questions to those who participated in the original survey. Follow-up questions could have explored further into the

behaviors and ideals of why participants consume electronic nicotine-based products. Finally, the *race* was another limitation that occurred in this study. There were not enough questions in the survey regarding race to conclude if the race had any relation to the young adult population and the consumption of electronic nicotine-base products. From previous research, it was found that minorities who lived in lower-income housing were targeted more frequently by tobacco companies compared to those who are White and live in high-income housing (Ickes et al., 2020; Luzius et al., 2020). For future research, race should be included in the study of this topic. Overall, future research on this topic could comprise an extended period to conduct follow-up questions for participants, a larger sample size that would include more male participants, and reassurance on the safety aspect for those who are distressed about the sensitive research topic.

In conclusion, the use of tobacco products will always be common in the world we live in. The global tobacco market was valued at $850 billion in 2021 and is anticipated to be over $888 billion in 2025 (Horowitz, 2022). This study examined how peer pressure does affect young adults. The hypothesis that was in relation to peer pressure was found to support the research and was tested using a chi-square crosstabulation. Although the study has some limitations, the information can be used and added for future research and exploration into electronic nicotine-based products.

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Development of a course-based undergraduate research experience (CURE) as a pedagogy to teach flow cytometric analysis on endospore formation in *Bacillus subtilis*

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Abstract

Bacteria must be able to adapt to environmental changes in their habitat. When conditions are harsh, certain bacteria will form dormant endospores in order to survive. Environmental stressors include nutrient deprivation. Endospores protect against high temperature, chemical disinfectants, desiccation, UV radiation, and gamma radiation. Endospore formation is restricted to only certain species of Gram-positive bacteria, such as *Bacillus* and *Clostridium.* This study used microscopic techniques and flow cytometry to investigate the endospore formation in the bacterium *Bacillus subtilis.* The characteristics, morphology, and viability of the bacterium were examined. The Schaeffer-Fulton method was used to dye the cells. SYTO-13™ dye solution was used for three cultures that were grown for 1, 8, and 13 days, and the flow cytometer determined the percentage of endospores versus vegetative cells in each population. Dot plots and histograms were created using forward scatter (FSC), side scatter (SSC) and FL – 1 fluorescence parameters. Day 13 had the highest proportion of endospores compared to day 1 and 8. After the new lab protocol was finalized, the background of endospore structure and formation, as well as the theory and application of flow cytometry were taught to Cabrini University's General Microbiology class as part of this course-based undergraduate research experience (CURE). The students took a pretest, and then performed the new lab procedure that was created for this course. The students were administered the same test to evaluate their improvement at the end of the semester. Results from pretest and posttest confirmed that the learning objectives of this lab exercise were effective. Future studies aim to focus on different environmental inducers and the effect on endospore formation in *B. subtilis*.

Introduction

Bacteria can persevere in many environments due to mechanisms of adaptation, one being sporulation. Bacterial endospores form in certain types of bacteria in response to unfavorable conditions or a lack of nutrients. In high levels of stress, these spores form in the bacteria through a process called sporulation. Spores have a high resistance to harmful environmental factors, such as heat, UV radiation, gamma radiation, chemical disinfectants, and desiccation. These spores can vary in size and location depending on the type of bacterium in which it forms (Abel-Santos, 2015). Spore structure is different than that of a normal cell

because it contains multiple layers in order to protect the genetic material of the bacterium. *Bacillus subtilis* is one of the most highly studied bacteria because of its cell density and sporulation response (Zheng et al., 2020).

Endospore-forming bacteria include human pathogens, which is why research is crucial to find treatment options for these diseases. An example is *Clostridium botulinum,* which causes a food-borne disease called botulism. This bacterium forms spores that are located subterminally, meaning they are located towards the ends of the cell. Another example is *Clostridium tetani,* an endospore-forming bacterium that causes tetanus. The spores are usually found in soil, dust, and manure, and are located terminally in a swollen sporangium (Popoff, 2020). Upon entering the body, the spores of *C. botulinum* and *C. tetani* germinate in oxygen-deficient tissues in the body and produce the exotoxins botulinum and tetanospasmin, respectively, which cause the physiological manifestations of the diseases. In this disease, the spores appear to be swollen in the sporangium. Although bacterial endospores can contribute to pathogenic diseases, their primary role is to protect the genetic material for survival, which is made possible by the numerous layers of the spore.

The coat of an endospore is made up of 70 proteins, and there are seven different layers found in an endospore (Willey et al, 2023). As illustrated in **Figure 1**, the outermost layer is the exosporium, followed by the coat, outer membrane, cortex, germ cell wall, inner membrane, and core. The spore's germ cell wall contains two layer of peptidoglycan, which is a large polymer that provides strength and inflexibility. In the core of the spore, there is a high concentration of dipicolonic acid complexed with calcium ions (Ca-DPA). These complexes are found between the nitrogenous bases of DNA and provide stability. The core also contains DNA, ribosomes, and enzymes with low water content. Spores are dormant structures that are metabolically

inactive and do not exhibit gene expression (Zheng et al., 2020). These factors allow a spore to survive for a long period of time without any available nutrients and in extreme environments. Once nutrients become unavailable, bacteria begin to form spores in a process known as sporulation.

Sporulation occurs when the growth of a bacterium ceases due to the lack of nutrients. This process occurs so that the bacterium can adapt to changes in the environment and survive. As seen in **Figure 2,** this process occurs in eight steps (Willey et al., 2023). The first step is called axial filament formation, which is when the DNA replicates and extends into the filament. Step two is septum formation, where the forespore and mother cell are separated with DNA remaining in both. In step three, the mother cell engulfs the forespore, creating another membrane. Step four is when the DNA in the mother cell begins to disintegrate. In step five, the forespore develops a layer of peptidoglycan between the forespore and mother cell. Step six is when the dipicolinic acid is synthesized and the spore coat is forming. Then, the endospore matures in step seven and becomes released by the mother cell in step eight. This cycle is regulated for the conversion of a metabolically active and dividing vegetative cell to a dormant endospore, but once nutrients are available, the endospores can revert back to vegetative cells.

The process from endospore to vegetative cell occurs in three steps (Willey et al., 2023). The first step is known as activation, where the spores prepare for germination. Then, germination occurs where the spore's dormant state is broken. Proteins, known as germinant receptors, detect small sugar and amino acid molecules. Once these are detected, the release of Ca – DPA complexes occurs, the breakdown of the peptidoglycan in the cortex begins and water is taken in. The third step is known as outgrowth, which takes place when water levels increase to that of a vegetative cell. Enzymes in the core also begin synthesizing various molecules that

are needed to begin spore outgrowth and return to a vegetative state. The processes from vegetative cell to endospore and back to vegetative cell can take place in many, but not all, Gram-positive bacteria, one of the most studied being *B. subtilis.*

B. subtilis, the bacterium used in this research study, is an excellent model organism for sporulation, germination, gene regulation, cell division, and cellular differentiation. Under a microscope, these bacteria appear as a straight rod with petritrichous flagella. *B. subtilis* forms spores that are subterminal. It is naturally found in environments containing soil and plant undergrowth, and is nonpathogenic to humans, plants, and animals. Spores are formed in the bacteria when there is a depletion of nutrients, such as carbon, nitrogen, and phosphorus (Beskrovnaya et al., 2021).

Flow cytometric analysis is often used in experiments to measure the amount of spores and vegetative cells found in a sample. According to Cronin and Wilkinson (2006), flow cytometric analysis can measure numerous parameters including the physiological state of each cell population, along with the heterogeneity of a bacterial population. The flow cytometer used in their experiment measured the percentage of germinating and outgrowing spores (Cronin & Wilkinson, 2006). Comas-Riu and Vives-Rego performed an experiment in 2002 to sort spores in samples of *Paenibacillus polymyxa* using flow cytometry. The results discovered by the use of the flow cytometry were promising, and researchers confirmed that the use of this technology is efficient and accurate (Comas-Riu & Vives-Rego, 2002). The use of flow cytometry is important for the statistical analysis of sporulation in different types of bacteria. The objective of this study was to develop a college-level, flow cytometry-based laboratory exercise to investigate spore production in *B. subtilis* by comparing young to old cultures with comparison to traditional staining methodologies. This course-based undergraduate research experience (CURE) was

implemented in the fall semester of 2022 for the BIO 308 – General Microbiology students at Cabrini University, and will be used again in fall 2023 in the same course with the introduction of additional parameters for students to investigate.

In previous years, students in BIO 308 used the traditional microscopic Schaeffer-Fulton method to stain one-week old cultures prepared in advance by the course instructor without having the ability to consider the effects of time on endospore formation or how to use flow cytometry as a tool to quantify endospore production. Implementing undergraduate research as a high impact practice in a CURE setting introduces students to critical and analytical skills beyond the cookie-cutter approach to traditional learning (Cooper et al., 2017). In this inquiry-based lab activity, students were not only able to research how extended incubation times affect induction of endosporulation, but they also examined whether flow cytometry methodology using cell permeant fluorescent dyes is an appropriate technique. Results of this CURE show that the procedure developed and the instrumentation used provide an accurate and reproducible means to differentiate vegetative cells from endospores, and to measure endospore formation as a function of time in a discovery-based format suitable for an undergraduate laboratory.

Materials & Methods

The formation of spores by *B. subtilis* was investigated by using numerous methods in order to optimize the protocol for use in the teaching laboratory. The first method tested the growth of *B. subtilis* on endospore agarose plates (ESA) and tryptic soy agar (TSA) plates. One plate for each medium was streaked out, and over the course of 10 days there were sixteen slides taken to examine under a microscope. These slides consisted of bacteria derived from colonies that were swiped from the ESA and TSA plate 1, 2, 3, 6, 7, 8, 9, and 10 days after *B. subtilis* was grown. These slides were stained using the Schaeffer-Fulton procedure, which stained the

endospores blue and the vegetative cells red. The Schaeffer-Fulton procedure started by placing a small piece of paper-towel over the heat-fixed bacterial samples on a glass slide, saturating it with malachite green dye, and steaming it for 5 minutes. The paper was removed, cooled, and rinsed with water for 30 seconds. The slide was then counterstained with safranin for 60 – 90 seconds, and then rinsed with water for 30 seconds. The slide was blotted dry with paper towels before observation at 1000x under oil using a Nikon compound light microscope. This method was used for all of the slides, and the proportion of vegetative cells versus endospores on each slide was established.

After determining that day 1 and 7 showed drastic differences between the amount of vegetative cells and spores, it was decided to look at these two days in further experiments. After different methods using varying amounts of SYTO-13™ dye, broths instead of agarose, and different methods of washing, the final revisions to the experiment were documented. Some complications that occurred during the process were the use of too much SYTO-13™ dye, clumps forming in the broth, and the amount of washing that occurred. Once these issues were resolved, the experiment was finalized and introduced to the BIO308 – General Microbiology lab course. The protocol used by the students is shown in **Protocol 1.**

An ESA plate was streaked out with *B. subtilis* onto three different plates over the course of 13 days to permit spore formation using cultures from 1 day, 8 days, and 13 days of growth. For each of the cultures days, samples were analyzed in triplicates by the three groups of students to facilitate statistical analyses. A sterile cotton swab was used to collect 5 large colonies of *B. subtilis* from each of the three ESA plates and transferred to 15 mL conical tubes that contained 5 mL of phosphate – buffered saline (PBS). The excess moisture from the swab was squeezed out onto the side of the tube, and the tube was capped securely. The tube was

vortexed on the Vortex Genie™ for one minute. The clumps settled for two minutes and 0.5 mL was transferred from the conical tube to a 1.5 mL microcentrifuge tube, making sure to avoid the clumps at the bottom of the tube. This was repeated with each of the three tubes to have triplicates of the microcentrifuge tubes for each of the three days. Three tubes were microcentrifuged at room temperature for 2 minutes at 5000 rpm. The supernatant fraction was discarded. After vortexing, the pellet was washed with 0.5 mL PBS and vortexed again for 10 seconds and the centrifugation and washing of each tube was repeated. Then, 0.5 mL of SYTO-13™ fluorescent dye (5μM) was added and the tube was capped and vortexed for 10 seconds followed by 5 min of incubation at room temperature, protected from light. After diluting with 0.75 mL PBS, the centrifugation and washing process was repeated twice more. The pellets were vortexed and resuspended in 0.5 mL PBS per tube and vortexed for 10 seconds. Each sample was then transferred to the flow cytometry tubes and stored in a dark container to avoid photobleaching. The samples were then run and analyzed using the BD FACSCalibur flow cytometer.

Using the flow cytometry protocol (**Protocol 2**), the nine samples were run using the flow cytometer using forward scatter (FSC), side scatter (SSC), and FL – 1 fluorescence parameters, and the data acquisition software BD CellQuest Pro that is interfaced within the flow cytometer. The flow was cleaned after use and tubes were discarded in the biohazard containers. The listmode data files were transferred to the appropriate storage device and analyzed using Verity WinList 9.0 software, according to **Protocol 3.** Histograms were generated using dual parameter (FSC vs. SSC) and single parameter (FL – 1) data.

Prior to this experiment, a microbiology class with 5 students was administered a pretest regarding the knowledge of *B. subtilis* and sporulation. The 20 multiple choice pretest/posttest

questions were designed in alignment with foundational information provided to students in the form of textbook/lab manual reading assignments, supplemental handouts, power point notes, and study guide questions covering sporulation and germination processes, as well as the theory and application of flow cytometric methodologies. After reviewing the theory of flow cytometry and the process of sporulation in pre-lab presentation notes, the students performed this lab experiment and they took the same test to measure if any increase in learning occurred.

Results

Schaeffer-Fulton Procedure

The Schaeffer-Fulton procedure was used to dye TSA and ESA slides with *B. subtilis* on days 1, 2, 3, 6, 7, 8, 9, and 10. After examining all 16 slides under a microscope (**Figure 3**), results showed that slides from the TSA plate showed more consistent results compared to slides from the ESA plate. After counting, day 2 on the TSA plate averaged about 0.667 spores among 50 vegetative cells**.** Day 6 had an average of 19.33 spores to 50 vegetative cells. However, on day 8, the average spores to vegetative cells increased to about 29.67 for every 50 entities counted. It is important to note that these values were determined by counting three randomly selected sections of cells found on each slide. To examine these values in a more precise manner, the flow cytometer was utilized.

Flow Cytometry

Four assays were conducted over several weeks in order to develop and optimize a protocol for use in the teaching lab. After making changes to the experiment, the fourth assay showed the most promising results. Our fifth assay incorporated Cabrini University's Microbiology students in the fall of 2022. Three plates were cultured for 1 day, 8 days, and 13 days in connection with scheduled lab days. After the samples were cultured and stained with

SYTO-13™, the flow cytometer was used to acquire the results from each day. **Figure 4** shows forward scatter (FSC) vs. side scatter (SSC) dot plots and Fl – 1 histograms for *B. subtilis* stained with SYTO-13™ at day 1, day 8, and day 13. Region 1 (R1) was used to capture the relevant cell population. The Fl – 1 histograms are gated on the R1 subpopulation. Region 2 (R2) represents the spore population, and region 3 (R3) represents the vegetative cell population in the histograms. The first row in **Figure 4 – part 1** is the no dye control, which, after gating, represented 14.19% of total events in the sample (R1). The second row shows day 1, where 0.07% of gated cells were spores, and 96.65% were vegetative cells. Row 1 of **Figure 4 – part 2** represents day 8, where 28.26% of gated cells were spores and 71.36% were vegetative cells. Row 2 of **Figure 4 – part 2** represents day 13, where 69.85% of gated cells were spores, and 29.94% were vegetative cells. An overlay of the four histograms that were depicted in **Figure 4,** providing a visual display of endospore presence over time, is shown in **Figure 5.** When comparing the three days on this overlay, it is evident that the number of endospores increases dramatically with time from day 1 to day 13. The students in the General Microbiology were able to create and analyze these figures based off of previous lessons taught to them involving the use of WinList 9.0 software for flow cytometric analysis.

General Microbiology Class

Two separate lessons were taught to the General Microbiology class at Cabrini University that had a class size of 5. The first lesson revolved around how the flow cytometer functions, and its purpose. The second lesson taught the class about the background of endospores, the process of sporulation, and how the Shaeffer-Fulton Method is performed. Before learning about either of these topics, the class was administered a pretest in order to assess their knowledge on the subject. After these lessons were taught, and the laboratory experiment was completed, the

students completed the posttest (same test) to evaluate their improvement. The grades from each of the five students and the class averages are shown in **Figure 6.** There were 20 multiple choice questions in the pretest/posttest, and the individual (**Figure 6A**) and average (**Figure 6B**) percentages of correctly answered questions was plotted in decimal format (0 – 1.0 representing 0% - 100% of correct responses, respectively). The pretest is available upon request; it is not provided in this paper because for consistency the same test will be administered to the fall 2023 class. The standard deviation for the class averages is depicted in **Figure 6B**.

The significance of these values was determined by running a student t-test, paired two samples for means. The p-value between the pretest and posttest was 0.0036. Since this number is < 0.05, there was a significant difference between the two exams demonstrating that student learning was successful.

Conclusion

In conclusion, the formation of endospores in Gram-positive bacteria is widely studied, and new techniques to analyze this concept are being developed to facilitate monitoring this process more effectively. Endospores form in response to unfavorable conditions, and can contribute to pathogenicity in humans. Research further allows us to gain more knowledge on the role of spore formation in virulence, and available treatment options when exposed to spore-forming bacteria. A crucial method to analyze the physiology and heterogeneity of endospores is flow cytometry. Analysis using flow cytometry was crucial in this research experiment, allowing the General Microbiology class to not only increase their understanding of the traditional Shaeffer-Fulton method, but also to gain exposure to the more advanced methodology of flow cytometry to investigate factors influencing sporulation in *B. subtilis.* The students established a clear and significant connection between culture time and spore formation in a closed system

where nutrient deprivation increased as the incubation period was extended. In addition, armed with the theory and application of flow cytometric techniques, the students are better equipped to conduct future lab experiments involving this methodology.

This lab exercise will be introduced again to students in General Microbiology in the fall of 2023 using the protocols presented in this study, with an extension built into this CURE activity that will investigate the effects of UV radiation, pH, and/or osmolarity on the sporulation process.

Acknowledgments

The authors of this work thank the Science Department at Cabrini University for providing the funding and facilities for carrying out this research project. Thanks are also extended to Jordan Brown, Leslie Hernandez, Triniti Heyward, Bryan Koshy, and Agaigeal Staniewski, the Fall 2022 students of BIO 308L – General Microbiology Lab, for participating in this CURE project and contributing to the research findings in this report.

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Appendix

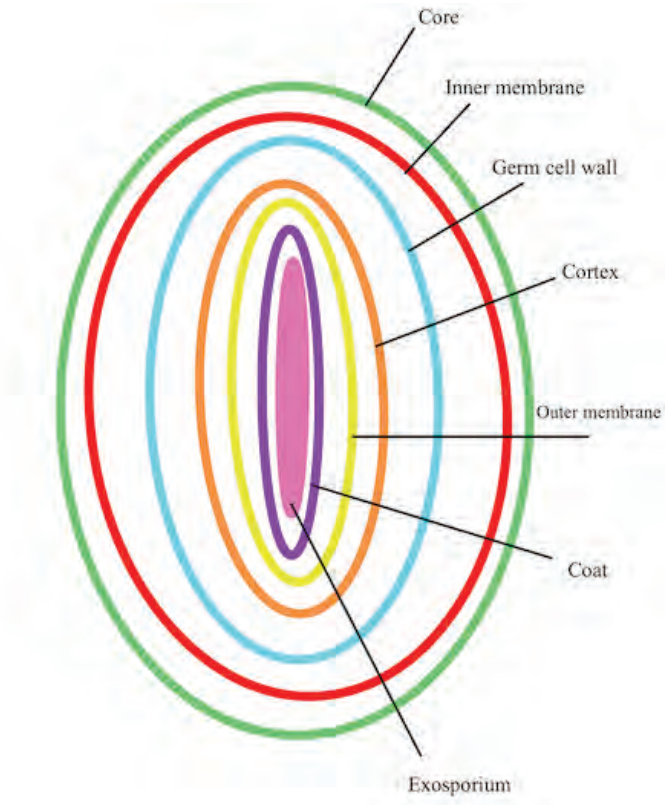


Figure 1. The seven layers of an endospore. This figure shows the seven layers that are found in an endospore. The core contains genetic material along with enzymes, and has low water content. The germ cell wall contains peptidoglycan, which works alongside the other layers to protect the genetic material in the core (adapted from Willey et al., 2023).

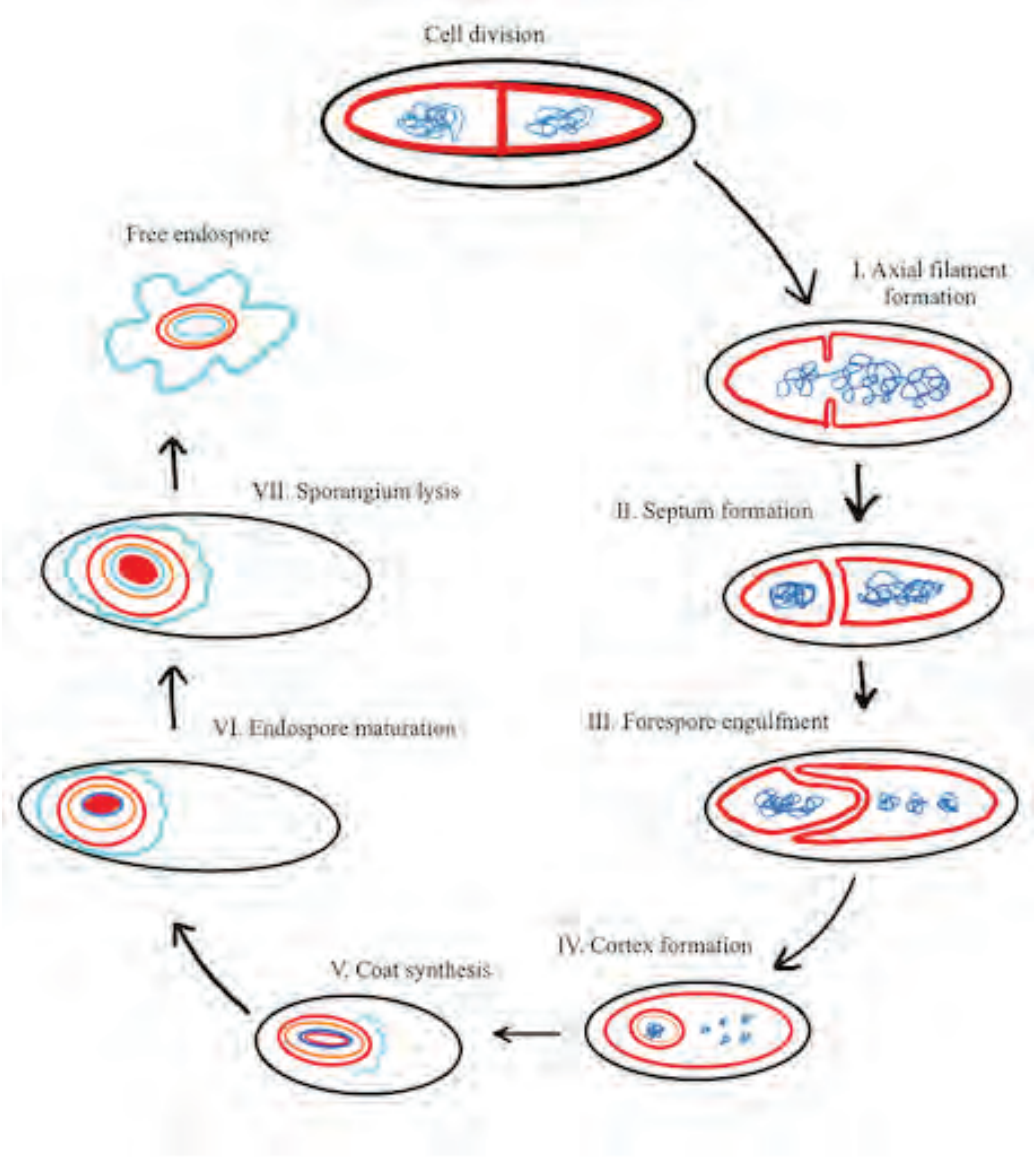


Figure 2. Process of sporulation. In this figure, the seven steps of sporulation are depicted, starting with axial filament formation, and ending with sporangium lysis (adapted from Willey et al., 2023).

Protocols

Using Flow Cytometry to Investigate the Effect of Long-term Culture on Endospore Formation in *Bacillus subtilis*

by Avery Byrnes and Sheryl Fuller-Espie, Cabrini University, November 2022

Protocol 1

To perform this laboratory experiment, three different student groups will use cultures of *Bacillus subtilis* that are 1, 8 and 13 days old. All cultures were grown on endospore agar (**Table 1**) at 30ºC, wrapped in plastic to avoid desiccation.

Table 1: Endospore agarose composition. After weighing out appropriate amount of sporulation broth, agarose was added for a final concentration of 1.5%. Media was autoclaved prior to plate preparation, and plates were stored at 4°C, wrapped in plastic, until needed.

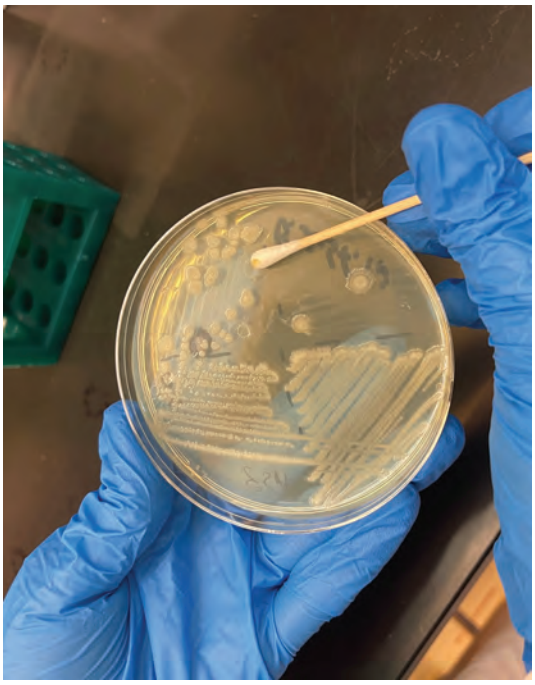
Ingredients	**g/liter**
Sporulation Broth: HiMedia Laboratories	
Peptone	6.00
Tryptone	4.00
Yeast Extract	3.00
HM peptone B #	1.50
Dextrose (Glucose)	1.00
Manganous sulphate	0.30
Agarose: SeaKem LE Agarose	15

Final pH (at 25°C) 6.6 +/- 0.2

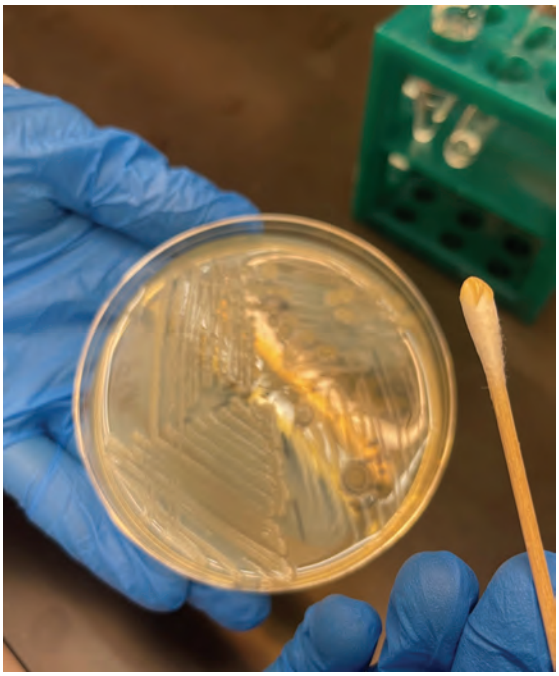
Materials Needed:
B. subtilis cultures: days 1, 8, and 13 on ESA plate (assigned depending on group number)
Sterile cotton swab
Phosphate-buffered saline (PBS)
1 15 mL conical tube
3 microcentrifuge tubes
3 flow cytometry tubes
Vortex Genie™
Microcentrifuge
Micropipetter (P1000)
500 mL glass beaker
BD FACSCalibur flow cytometer
SYTO-13™ green fluorescent nucleic acid stain – 5 mM stock solution in DMSO (used at 5 μM in PBS in staining procedure)

Laboratory Experiment:

1. Using a sterile cotton swab, collect 5 large colonies of *B. subtilis* from the ESA plate and transfer cells to a 15 mL conical tube containing 5 mL PBS, being sure to squeeze out the excess moisture from the swab on the side of the tube, and capping the tube securely.



A



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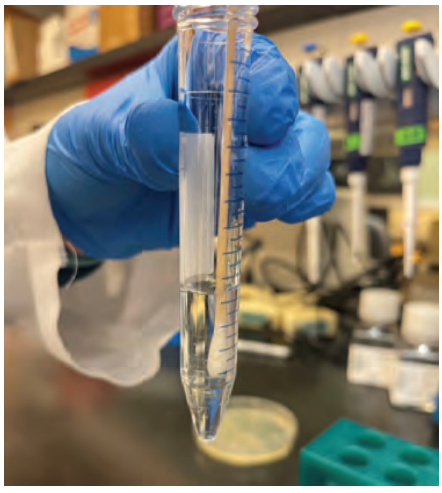

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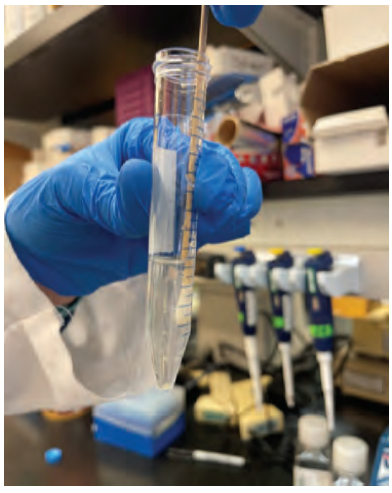

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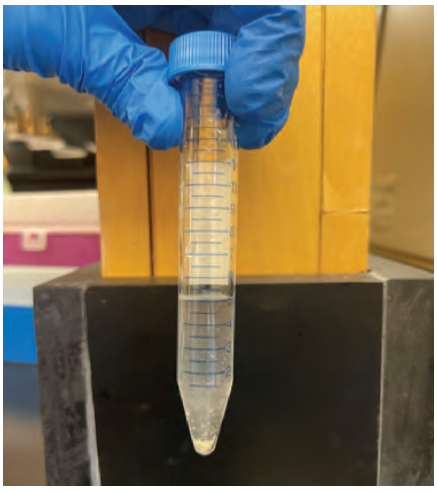
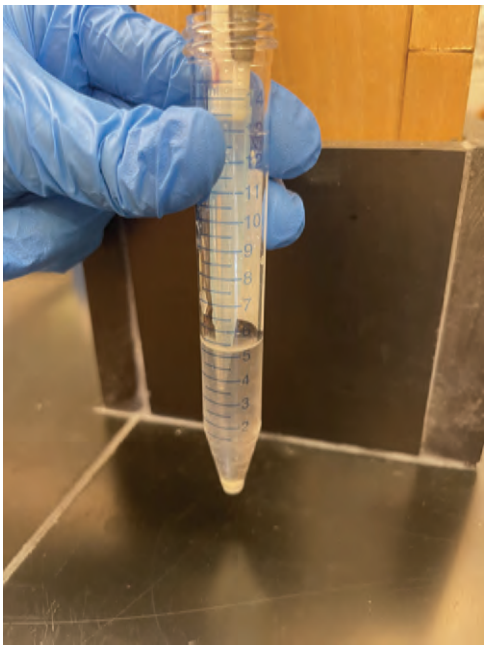


E

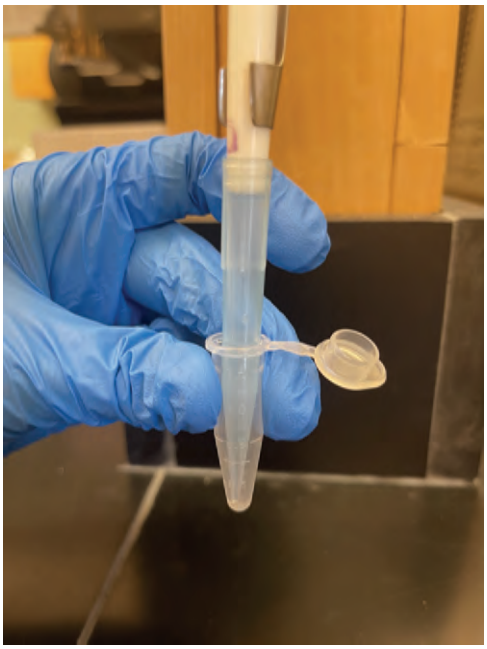
Protocol Figure 1: Retrieval of *B. subtilis* from ESA plates. Figure 1A shows the collection of 5 colonies from the plate. Figure 1B is after the collection of colonies, seen on the cotton

swab. Figure 1C shows the cotton tube being placed in the conical tube with the PBS. Figure 1D shows the cotton swab being moved around in the tube to release the colonies. Figure 1E is the result after the colonies being transferred to the conical tube, settling to the bottom of the tube.

2. Vortex the tube on high for one min.
3. Allow all clumps to settle to the bottom of the tube for 2 min.
4. Transfer 0.5 mL from the conical tube to a 1.5 mL microcentrifuge tube, making sure to avoid the clumps that have settled to the bottom of the tube.



A

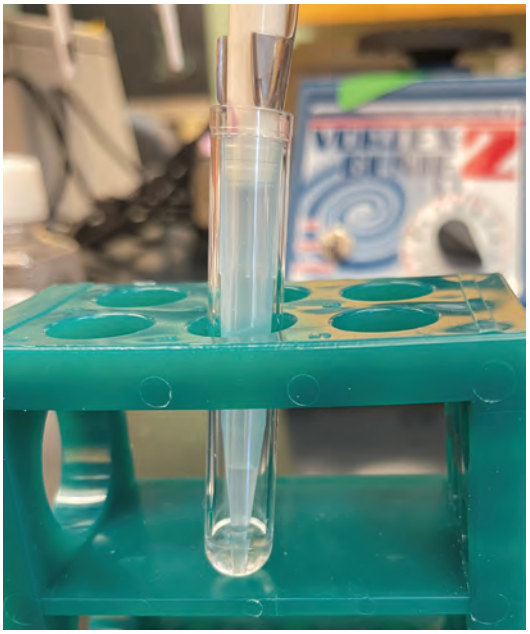


B

Protocol Figure 2: Transfer of suspended *B. subtilis* to microcentrifuge tube. Figure 2A shows the collection of 0.5 mL from the conical tube using a P1000 micropipetter, only collecting the top portion of the sample. Figure 2B shows the transfer into the microcentrifuge tube, making sure the tip is placed at the bottom of the tube to avoid getting the sample on the sides.

5. Repeat step 4 two more times in order to have 3 microcentrifuge tubes (triplicates).
6. Spin all 3 tubes in a microcentrifuge at room temperature for 2 min at 5000 rpm. Orient your tubes so that the hinge of the tube is furthest from the center of the rotor. After centrifugation, inspect for a visible pellet at the bottom of the tube on the hinge side, and then carefully and thoroughly decant supernatant into the glass beaker by inverting the tube and flicking the liquid content of the tube into the beaker. Vortex the tube for 10 seconds.
7. Add 0.5 mL PBS to wash the pellet, cap the tube securely, and vortex for 10 seconds.
8. Repeat step 6.
9. Add 0.5 mL SYTO-13™ fluorescent dye to resuspend the pellet in each tube, cap the tube securely and vortex 10 seconds. Incubate for 5 minutes, protected from light.

10. Dilute the sample by adding 0.75 mL PBS and vortex for 10 sec.
11. Repeat steps 6-8.
12. Resuspend the pellets in 0.5 mL PBS per tube. Vortex 10 sec. Very carefully transfer 0.5 mL of samples to flow cytometry tubes, placing the tip of the micropipettor at the bottom of the flow cytometry tube before slowly releasing the sample into the tube (Figure 3).



Protocol Figure 3: Transfer of stained cells to flow cytometry tubes. The image above shows the transfer of cells to the flow cytometer tube, making sure to place the tip of the micropipetter at the bottom of the tube to ensure that none of the sample will splatter on the sides of the tub.

13. Store samples protected from light until needed for Protocol 2.

Protocol 2

1. Store samples protected from light until needed for step 14.
2. Run samples on the BD FACSCalibur flow cytometer using forward scatter (FSC), side scatter (SSC), and FL – 1 fluorescence parameters, and the data acquisition software BD CellQuest Pro that is interfaced with the flow cytometer.
3. Clean the flow cytometer after use and dispose of all tubes in the biohazard container provided.
4. Transfer listmode data files to an appropriate storage device.
5. Analyze listmode files using Verity WinList 9.0 software. Prepare dual parameter (FSC vs SSC) and single parameter (FL-1) histograms, gating the FL-1 histogram on a region of cells exhibiting appropriate FSC and SSC properties as instructed in class, and shown in Protocol 3 below.

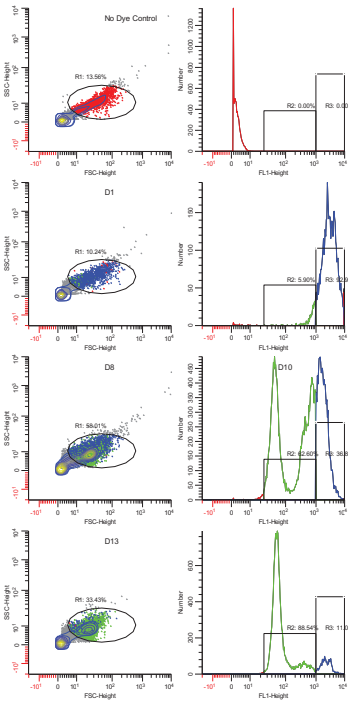
Protocol 3

Endospore Formation in *Bacillus subtilis*: A Quantitative Study Using Flow Cytometry
BIO 308 – General Microbiology – Fall 2022
WinList 9.0 Software Instructions:

1. Transfer Listmode file from thumb-drive to desktop; drag and drop 'Microbiology 2022' folder to desktop. Open and minimize a MSWord document and your email account.
2. Launch WinList 9.0 Application: Search applications for WinList 3D 9.0.1, click 'OK' after launching and enlarge window to full size.
3. On the Navigation Menu Bar at the top, click **OPEN FCS**. Navigate to the 'Microbiology 2022' folder on the desktop, open the 11-30-2022 Endospore Exercise folder, and click/open Data 001 (this is the no dye control file).
4. The 'Create Histogram' box will pop up. For the two parameter (2P) dot plot, select FSC-H (x-axis) and SSC-H (y-axis) and add to list. For one parameter (1P) histogram select FL-1H and add to list. Click OK.
5. Draw Region 1: Click anywhere on the 2P dot plot. This will cause the right-hand navigation bar within that plot to appear. Select the 4th tool down (Ellipse) and apply region to the desired population of cells (as demonstrated in class). Adjust the region's size, then reposition and adjust its label.
   Note: it's very easy to add extra regions. If this happens, click on the R1=R2= button in the navigation bar, select a region for elimination, and press 'delete.' Select that same region again and press 'clear', then click OK.
6. Gate FL-1H histogram on R1: Click R1=R2= button at top of window. An 'Edit Gates' box will pop up. Press 'Reset All'. Highlight the G1/R1 line, then double click on the heading of FL-1H histogram and the title of the histogram will subsequently change. The title will now be 'FL-1H [G1=R1]'. Finally, click 'OK' in the 'Edit Gates' box to apply changes and the appearance of the histogram will automatically update.
7. Set two new regions to quantify % of endospores: To do this, select file 10 (13 days of growth on endospore agar) from Listmode files in folder by:
   a. Clicking on '**Next FCS**' (NOT OPEN FCS!!!), select Data 010, and OPEN. For the purpose of this exercise, vegetative cells (which readily take up the cell permeant dye SYTO-13™) will be the very far-right curve on the graph, whereas everything else will be counted as endospores.
   b. To Create R2: Click anywhere in FL-1H histogram. Select 2nd tool from top from right-hand tools. Place cursor immediately to left of first curve on the left and extend to cover first two peaks on the graph, but not the third peak.
   c. Create R3: Select 2nd tool again. Create another region starting at the right side of R2 and extending almost to the end of log scale. It is important that the two lines are aligned precisely – this may take a little bit of manipulation, but once it is set, it will not need any further adjustment for the remainder of the analysis.

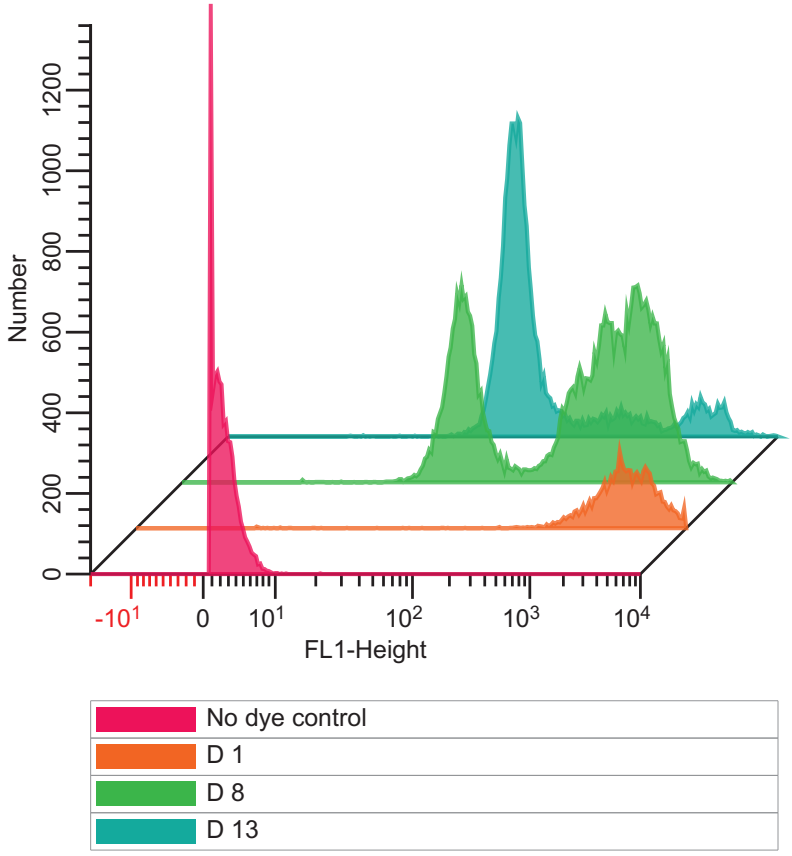
d. Adjust labels as instructed in class; click on the dot, not on the label, and drag to desired location above the regions boxes.

8. Create multigraphs: Use files 1, 4, 7, and 10:
    a. Return to File 001 by selecting '**Next FCS**' and clicking on Data001. Then click on 'Multigraph' from top menu bar – and an 'Edit Multigraph' window will open. Highlight only Data 001 FSC vs. SSC and Data 001 FL-1H (NOT 'Blank') and click 'Add to Multigraph Items.'
    b. Add graph labels for FSC vs. SSC (No Dye Contro) and remove any labels for FL-1H as instructed in class.
    c. At the bottom left, select 'Tiled, 2 columns, 4 rows.' Click 'OK.'
    d. Repeat with files 4, 7, and 10 – adding labels D1, D8, and D13, respectively. To do this, click on '**Next FCS**', Data004, and open. Then click on heading of multigraph, highlight relevant files, and as before, change labels and click 'ok'. Repeat for files 7 and 10.
    e. When finished, copy multigraph to a word document. Your completed Multigraph will look like this if completed correctly:



9. Create an overlay:
    a. First, remove regions 2 and 3. Refer to step 5 above on how to remove a region. Click on '**Next FCS'** and return to Data 001. Then click on 'Overlay' tab on top menu bar, and an empty window will open.
    b. Using the cursor, click on top left histogram icon on FL-H histogram and drag and drop to the overlay window.
    c. Change the label: right-click on Data001 label, select Edit, then select Data001 on the far-left side. Finally, change the label to No Dye Control, and click 'OK'.

d. Three more histograms will be overlayed on top of this one. Use files 4, 7, and 10 as demonstrated in class. Be sure to change labels to D1, D8, and D13 respectively.
e. Copy and paste the overlay into your MSWord document. Send the MSWord document to yourself using your email account or an appropriate storage device.
f. If completed correctly, your overlay will look like this:



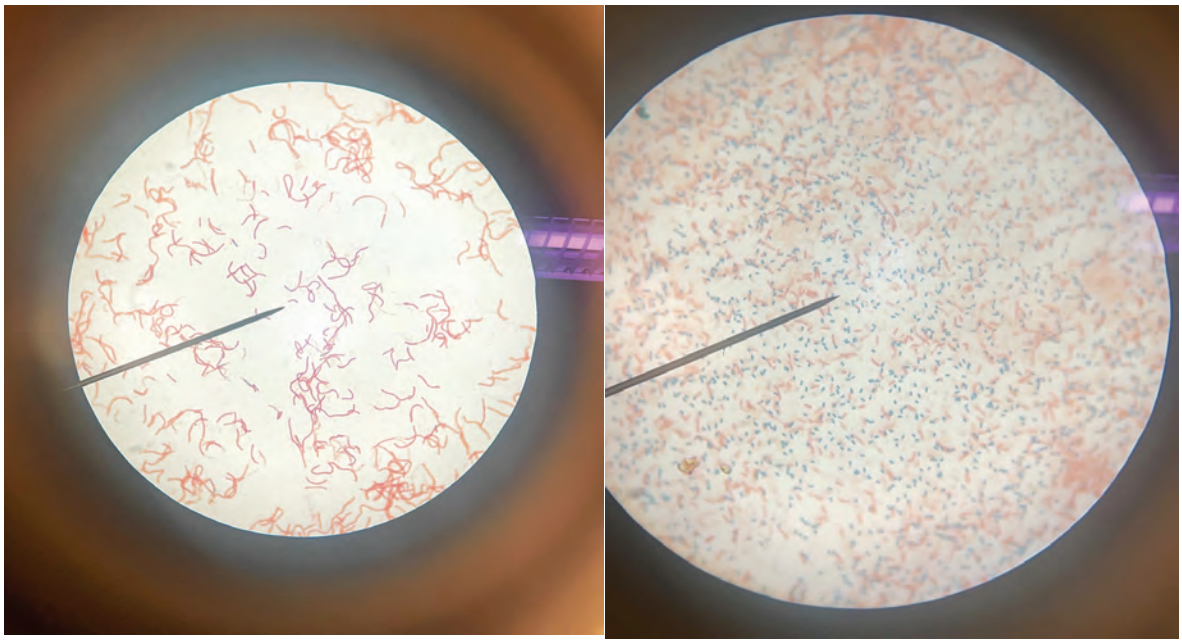


Figure 3: Day 2 & 8 TSA slides under microscope. These images were taken through the lens of a microscope to evaluate the number of vegetative cells (red) vs. endospores (green) formed on a TSA plate that was streaked out with *B. subtilis* and incubated for 2 days and 8 days. There are many vegetative cells and little spores in the day 2 slide, compared to many spores and little vegetative cells in the day 8 slide.

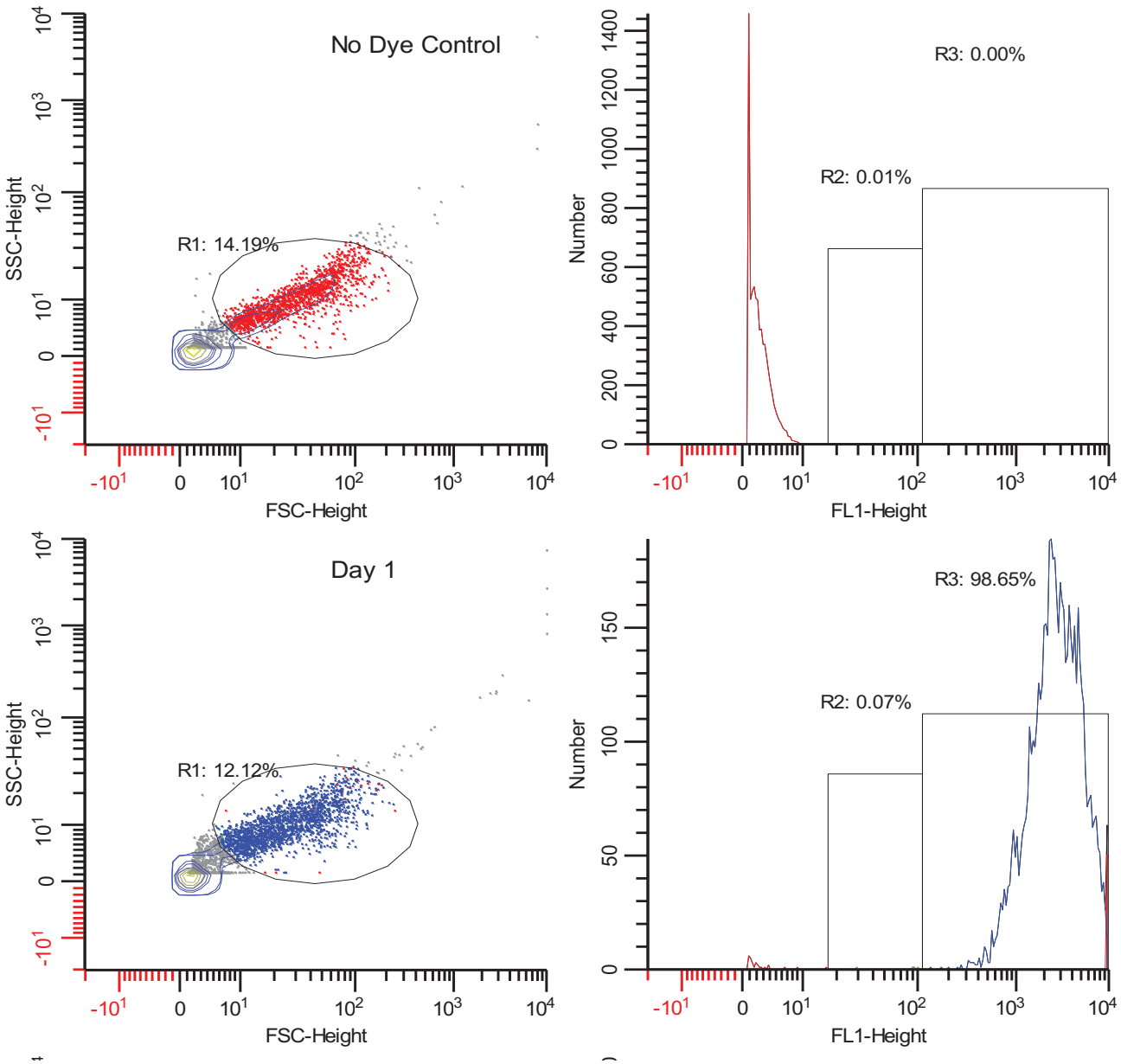


Figure 4 – part 1: Representative dot plots and histograms acquired by the flow cytometer. The x-axis represent forward scatter (FCS) and the y-axis represents side scatter (SSC). The first dot plot is the no dye control, and the second dot plot is for *B. subtilis* stained with SYTO-13™ at day 1. Region 1 (R1) was captured to obtain the relevant cell population. Region 2 (R2) represents the spore population and region 3 (R3) represents the vegetative cell population. For the day 1 dot plot, 0.07% of spores made up to R1 and 98.65% of vegetative cells were the remaining cells in R1.

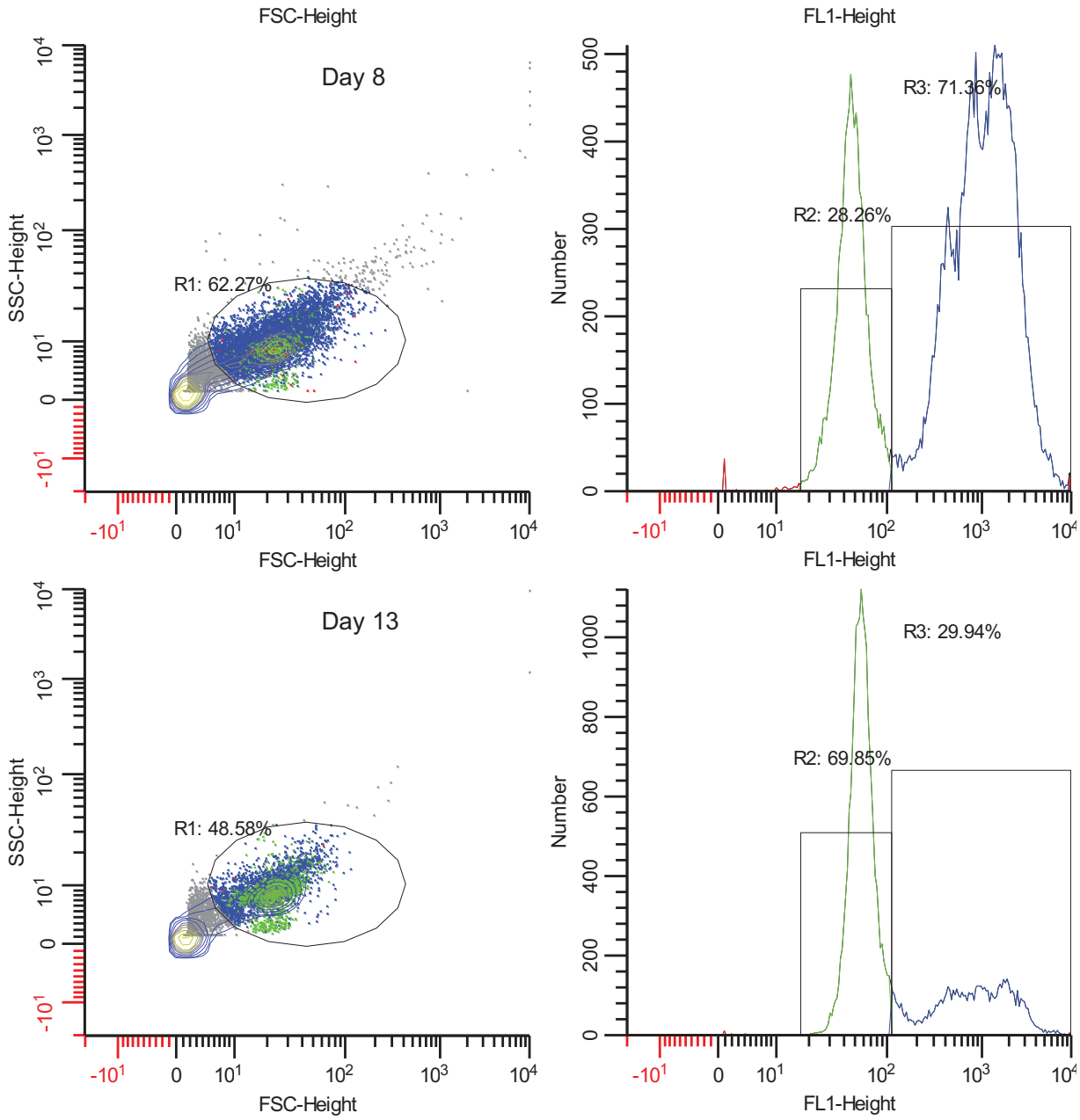


Figure 4 – part 2: Day 8 and day 13 results. Histograms show that 28.26% of spores make up R1, and 71.36% accounts for the remaining cells in R1. As for day 13 results, 69.85% of cells in R1 are spores and 29.94% of cells in R1 are vegetative cells.

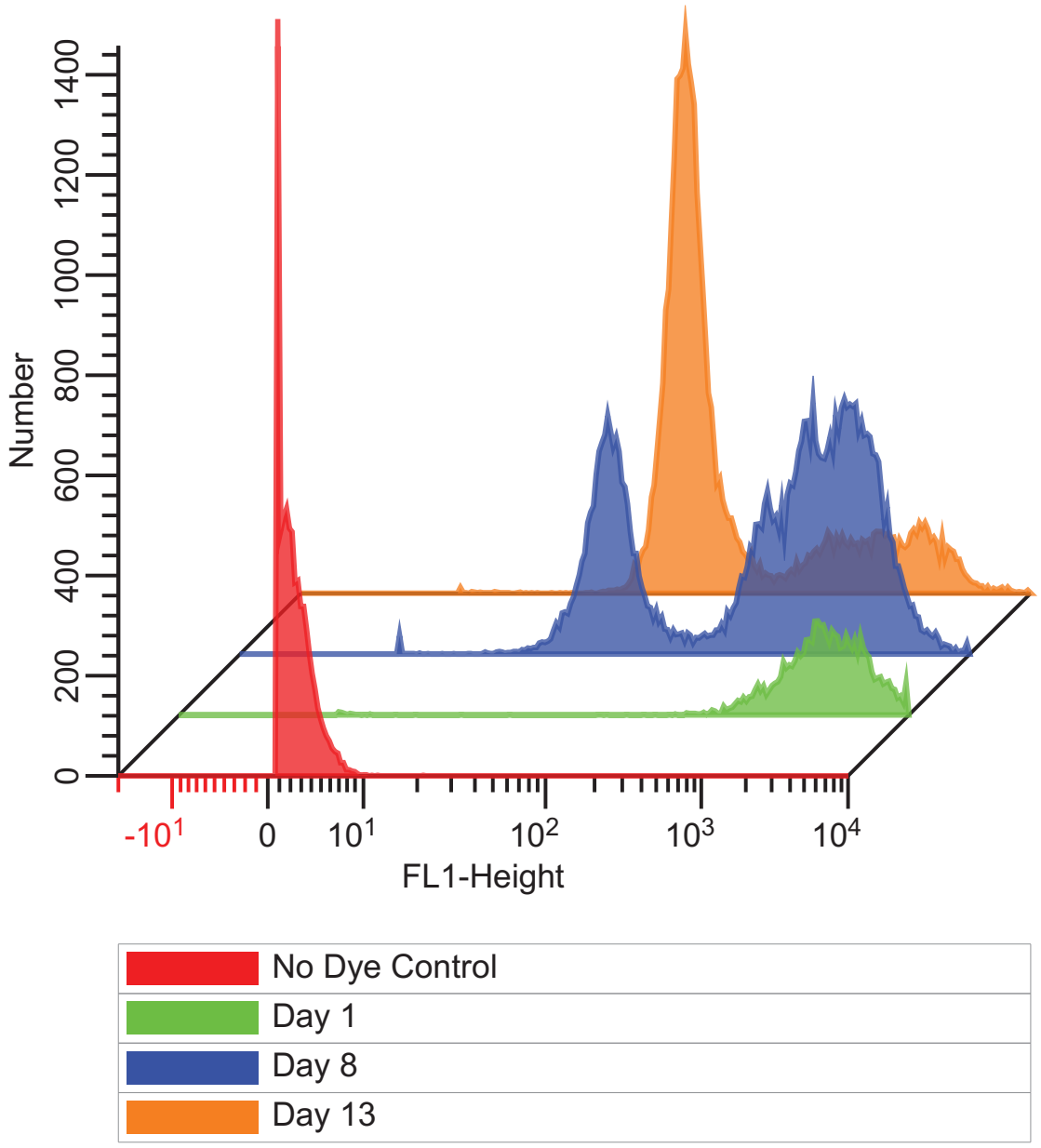
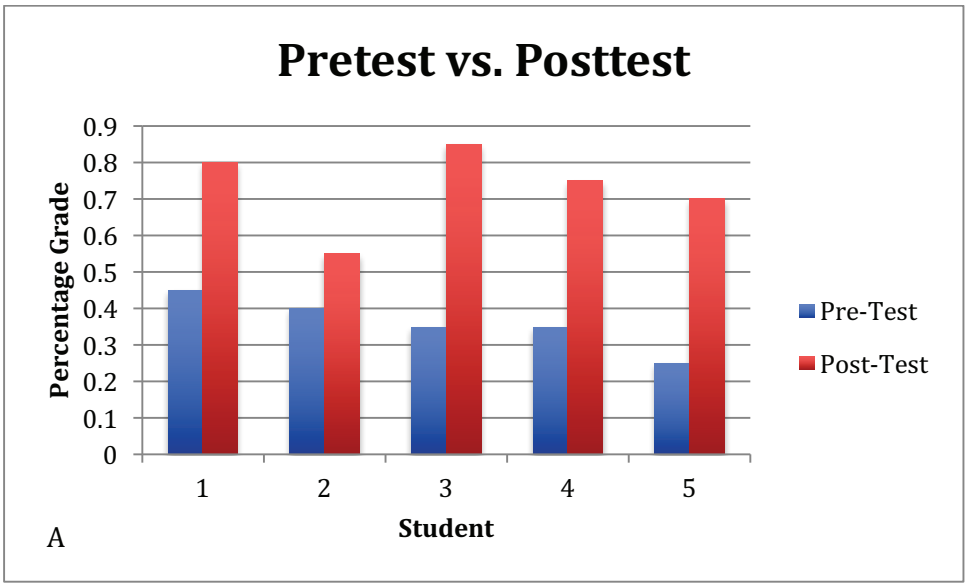


Figure 5: Representative overlay from four histograms obtained by WinList Software. The no dye control is the first histogram, day 1 is the second, day 8 is the third, and day 13 is the fourth. No cells were dyed in the no dye control. Day 1 shows a miniscule percentage of spores, and mainly vegetative cells. Day 8 contains more spores than day 1, but still has more vegetative cells. Day 13 has a significant increase in the number of spores compared to vegetative cells.



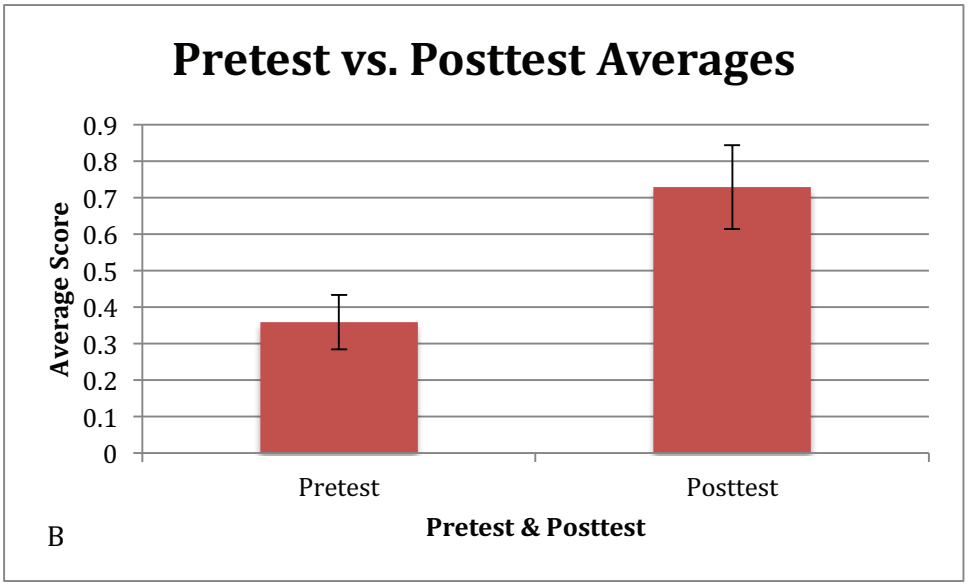


Figure 6: Pretest and posttest results from students enrolled in Microbiology, fall 2022. Part A results show that each student had increased their score in their posttest compared to the pretest. After running a student t-test, the significance value was 0.00360102. Since this value is <0.05, there was a statistically significant difference between the average test results in the pretest and posttest. Part B shows the averages from the pretest and posttest, along with the standard deviations bars that had values of 0.074 and 0.115, respectively.

Anxiety, Depression, and Substance Use in Undergraduate Students

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Abstract

Since everyone is a stakeholder in the higher education system in some way, it is necessary to be concerned about the mental and physical health of today's undergraduate students. It builds upon prior research that has looked at mental health in undergraduate students and numerous studies that have investigated substance use habits in undergraduate students. The current study aims to investigate the relationship between students with anxiety and/or depression symptoms and their substance use habits. It gathers quantitative data through availability sampling with an electronic survey of 79 Cabrini University students in the Spring 2023 semester. It asks about the students' anxiety symptoms, depression symptoms, alcohol use, and drug use. The researcher hypothesizes that students who experience generalized anxiety symptoms are more likely to be substance misusers than those with fewer and/or less severe anxiety symptoms. Additionally, it is hypothesized that students who experience depressive symptoms are more likely to be substance misusers than those with fewer and/or less severe depressive symptoms. Results from Spearman correlations show a significant relationship between anxiety symptom frequency and marijuana use; however, no other correlations are found between the other anxiety, depression, alcohol, and drug misuse variables test.

Introduction

The higher education system in America plays a pivotal role in society's future. Even people who are not in college themselves are still stakeholders in the education system in some way. Whether it is companies looking to hire young professionals, schools looking for future educators, or advancements being made in the medical field, various societal factors rely on the higher education system (Habibi et al., 2014). Because of this, everyone depends on colleges and the success of college students. Preserving the health and well-being of today's youth is a crucial step in ensuring a successful future for the country (Habibi et al., 2014). Mental health and substance use in college students are some of the most influential factors in understanding the well-being of today's undergraduates.

Mental health is one of the most pressing problems among undergraduates today. Of the countless disorders people can endure, generalized anxiety and depression are unquestionably the most prevalent issues for undergraduate students (Kraft et al., 2021). About 37% of college students suffer from depression, as defined by the Diagnostic and Statistical Manual of Mental

Disorders, Fifth Edition (DSM-5), and 48% of college students suffer from anxiety (Kraft et al., 2021; Podina et al., 2022). Generalized anxiety is defined as excessive worry and apprehensiveness; depression is defined as a diminished interest/pleasure in activities that once brought joy (American Psychiatric Association [APA], 2013). Statistics of individuals suffering from anxiety and depression symptoms are shockingly high, and rates continuing to rise with the growing acceptance and awareness of mental health issues. It is crucial to understand that other factors play an influential role in the mental health of college students, as well, such as alcohol and drug use.

Substance use is a growing epidemic in today's colleges and universities. As of 2020, 60% of undergraduate students had consumed alcohol, and 39% had engaged in binge drinking (Pedrelli et al., 2020). Per the Centers for Disease Control and Prevention (CDC), binge drinking is defined as five or more drinks in two hours for males or four or more drinks in two hours for females (Centers for Disease Control and Prevention [CDC], 2019). Additionally, about 45% of 21-year-olds have used marijuana in the past year, and 18% of college students have used illicit drugs (not including marijuana/cannabis) (Schulenberg et al., 2020). With over half of college students engaging in binge drinking and almost half using marijuana/cannabis products, substance abuse is extremely prevalent today. From looking at these statistics, it is evident that alcohol and drugs have almost become an expected part of college life.

The U.S. Department of Health and Human Services (HHS, 2022) found 1,519 college students died from alcohol-related causes in 2021. This included unintentional injuries as well as motor vehicle crashes related to alcohol intoxication (HHS, 2022). It is difficult to estimate the true number of drug-related deaths since drugs are often used in conjunction with alcohol and get

conflated into alcohol-related statistics (HHS, 2022). However, there is no reason any college student should suffer this death, and research is the first step in preventing further tragedy.

Investigating the relationship between anxiety and depression symptoms with substance use in college students is an important part of solving this mental health epidemic. Self-reported surveys, such as this study, are just one way researchers can explain the underlying connections between mental health and substance use. With conclusions from these studies, researchers can cooperate with undergraduate institutions to implement more effective programs and policies. And since college students are the future of society, it is important to create a safe environment that supports their physical and mental well-being.

Literature Review

Anxiety and Depression

Importance

Mental health issues have always been present in society; however, the DSM and its various editions have contributed to the growing awareness and acceptance of these disorders (Kraft et al., 2021). Until recently, many people thought anxiety and depression were not real; people claimed this was a way to gain attention, drug seeking, and not take responsibility for their actions (Habibi et al., 2014). In the past, sufferers of anxiety and depression were expected to 'tough it out,' or if their symptoms were severe enough, they were deemed crazy and referred to a mental health hospital (Preonas & Lau-Barraco, 2021). Recently, society has adopted a more rehabilitative perspective that focuses on the medicalization and treatment of mental disorders with the belief that they can be alleviated and possibly even cured (Habibi et al., 2014). Once people have been diagnosed, they can take medication, attend therapy, join support groups, and

work toward living a healthier life (Kraft et al., 2021). Though even with numerous treatment options available, there remains a great stigma surrounding mental health issues.

Prevalence

Despite the staggering anxiety and depression rates, there are still an overwhelming number of people who do not seek treatment, even when experiencing the symptoms described in the DSM-5 (Podina et al., 2022). People are often scared of the stigmatization and negative views of being diagnosed that come with being diagnosed with a mental disorder, or they may not even realize their symptoms could fall under the classifications of a mental disorder (Kraft et al., 2021). It is estimated that about 30% of people that meet the criteria for having a form of anxiety or depression, as defined by the DSM-5, never seek treatment for it (Podina et al., 2022). As for college students, anxiety and depression are undoubtedly the most common mental health issues (Habibi et al., 2014). Many researchers believe the prevalence of these disorders in college students is so high because this is such a stressful, emotional, confusing, and ever-changing time in people's lives (Kraft et al., 2021). This stress is commonly self-medicated with alcohol and drug use by students (Bravo & Pearson, 2017).

Substance Use

Alcohol consumption

The dangers of alcohol consumption become most critical during someone's college career because alcohol is so common in this setting (Pedrelli et al., 2020). There are various factors that contribute to the prevalence and potential threat that alcohol can pose for undergraduate students. First, the abundance of social events and parties that involve drinking is thought to play a large role in why college students have such high rates of alcohol consumption (Pedrelli et al., 2020). Students gather and drink alcohol for birthdays, tailgates, parties, and

casual hangouts (Preonas & Lau-Barraco, 2021). Many self-reported studies even show that students look for occasions to drink (Saini & Suthar, 2022). This may act as a neutralization technique so that students do not feel guilt for their excessive binge drinking habits but instead claim that there is an excuse to drink (Pedrelli et al., 2020).

While at these gatherings, the role of peer pressure comes into play, which contributes to the staggering rates of binge drinking among college students as well. Because there are so many people who drink alcohol in college, it is hard to stay away from it (Pedrelli et al., 2020). Even those who may not have the desire to drink will likely feel pressured by peers since most social gatherings involve alcohol consumption (Saini & Suthar, 2022). This pressure can be overt, where the peers outwardly encourage others to drink, or covert, where a person internally feels pressured to drink just because everyone else is (Preonas & Lau-Barraco, 2021). Even underaged students drink, and it is considered the "normal" thing to do, despite being illegal (Saini & Suthar, 2022).

The presence of alcohol and peer pressure is a dangerous combination for undergraduate students since heavy alcohol consumption is encouraged. Peer pressure could foster a student's drinking habits and influence them to drink an unsafe amount (Saini & Suthar, 2022). Height, weight, gender, hydration, food consumption, time elapsed, and the type of alcohol can all influence how severely the effects of alcohol impact the drinker (Pedrelli et al., 2020). Because of this, everyone's limit of how much is safe to drink is different. Encouraging someone to drink past their limit could lead to severe immediate health concerns, which include vomiting, distorted vision/hearing, breathing difficulties, impaired judgment, decreased depth perception, impaired coordination, blackouts, or even coma (Substance Abuse and Mental Health Services Administration [SAMHSA], 2022). Alcohol has also been shown to increase aggression and risk-

taking behavior, which could lead to physical violence, drunk driving, getting hit by a car, drowning, or a life-threatening fall (SAMHSA, 2022). These are unarguably serious incidents that could severely threaten someone's life. Next, it is important to understand drug use, both independently and in conjunction with alcohol use. Alcohol abuse alone is dangerous, but when used simultaneously with drugs, the results can be even more deadly.

Drug use

Although drug use is more widely accepted because of the rehabilitative era, researchers still have similar pressing concerns with drug use as they do for alcohol use (Prosek et al., 2018). The rehabilitative era has opened many people up to the idea of looking for new resolutions as an alternative to incarceration. This often includes prescription drugs as a remedy when necessary for an offender (Prosek et al., 2018). With almost half of students using marijuana and almost a fifth using other illicit drugs, substance use is far more common in college students than people may realize (Schulenberg et al., 2020). The debate over the long-term effects of marijuana is extensive, with some research showing that marijuana usage can lead to permanent cognitive impairment, increased anxiety, excessive paranoia, and a higher risk of psychosis; conversely, other research has shown that marijuana may have little to no long term mental or physical consequences for users (Prosek et al., 2018).

As for other illicit drugs, most researchers agree that these are dangerous and even life-threatening, as drug use can lead to addiction, overdose, and permanent cognitive impairment (SAMHSA, 2022). Opioids, prescription medication, Adderall, Ritalin, ecstasy, MDMA, LSD, cocaine, and heroin abuse are common on college campuses, which can lead to memory loss, confusion, decreased motor skills, frontal lobe damage, liver damage, heart disease, lung disease, cancer, and more (SAMHSA, 2022). Medicalization refers to the view that much of society has

adopted, where prescription drugs are used as a remedy for both mental and physical ailments or disabilities (Prosek et al., 2018). While this perspective has been beneficial for many, the abuse of drugs is a growing problem since the medicalization era promotes drug use, which can become misused (Prosek et al., 2018). One of the most common rehabilitative methods for both mental and physical ailments is medication (Schulenberg et al., 2020). With the start of the opioid epidemic in the 90s, drug production, distribution, and consumption have increased drastically (Prosek et al., 2018). In many cases, medication can be a safe and effective solution to a mental or physical issue, but not everyone can handle intense drug use without becoming addicted (Schulenberg et al., 2020).

Even if someone does not become addicted to these substances, the increased production and distribution of prescription drugs and opioids have allowed for much easier access to drugs; this poses the threat that these substances are more likely to fall into the hands of unintended users, such as college students (Prosek et al., 2018). Next, it is crucial to look at how these mental health and substance use factors connect.

Substance Use and Mental Health

To summarize, mental health and substance use are extremely prevalent and dangerous in today's undergraduate population. The increase in responsibilities is a driving factor in the spike in anxiety and depression cases in college students (Saini & Suthar, 2022). During college, students often gain several responsibilities (renting a home/apartment, living on their own, paying for school, sports/extracurriculars, classes, etc.), which can cause excessive stress and worry (Saini & Suthar, 2022). Many students struggle with this newfound freedom and the duties that come with it, so they use alcohol and drugs as a way to cope and adjust (Bravo & Pearson, 2017). Substance use becomes a way for students to relax and temporarily forget these

overwhelming obligations (Saini & Suthar, 2022). However, this can escalate into a dependency on substances to function. Increased substance use can lead to more severe anxiety and depression symptoms, which can promote more substance use to the point where the two variables are codependent and encourage each other (Saini & Suthar, 2022).

When students turn to alcohol and drugs to relieve anxiety and depression symptoms, it can lead to addiction (Bravo & Pearson, 2017). Whether prescribed by a doctor or not, students can become addicted to medication to cope with the stressors of everyday life (Saini & Suthar, 2022). Because opioids are so commonly used as a treatment for medical or physical ailments, there is an increased production and accessibility to these drugs (Saini & Suthar, 2022). This increased access to drugs allows prescription medication to be abused or passed along to others, which contributes to the growing substance use rates in college students (Bravo & Pearson, 2017).

With all this information, it is imperative to continue research on mental health and substance use in college students, so the nuances of the relationship can be understood. Anxiety, depression, binge drinking, and drug use will never fully disappear; however, society must acknowledge the problem and take action. If everyone collaborates and supports policy/program change, this debilitating epidemic can be diminished.

Hypotheses

1. Students who experience generalized anxiety symptoms are more likely to binge drink or misuse drugs than those with fewer/less severe symptoms.
2. Students who experience depressive symptoms are more likely to binge drink or misuse drugs than those with fewer/less severe symptoms.

Methodology

Sample and Procedure

A nonprobability, availability sampling method was used to collect the data for this study. Data was collected from N=79 participants from Cabrini University. Self-administered electronic surveys were distributed to Cabrini University students in the spring of 2023. Professors in the Sociology, Criminology, and Justice Department were contacted through email and asked to distribute the survey link to their classes. Survey links that were sent to students were available for the students to complete voluntarily at their own convenience. The researchers also distributed the survey link through emails and text messages to friends, peers, and classmates.

The survey was voluntary, and students signed an informed consent before completing the survey. This form included the purpose, risks, benefits, and the participants' rights to contact the primary researcher with any comments or concerns. Additionally, the study and survey were approved by the Institutional Review Board (IRB) at Cabrini University before being distributed to students.

A total of eighty-four surveys were administered; however, five responses had to be thrown out after data cleaning because the respondents were graduate students, which the survey was not approved to be administered to; as such, N=79 respondents remained in the dataset. In terms of gender identity, the sample consisted of 73.4% who identified as female (n=58), 25.3% who identified as male (n=20), and 1.3% who identified as cisgender (n=1). As for the age distribution, 13.9% were 18-year-olds (n=11), 26.6% were 19-year-olds (n=21), 15.2% were 20-year-olds (n=12), 31.6% were 21-year-olds (n=25), 8.9% were 22-year-olds (n=7), 1.3% were

23-year-olds (n=1), and 2.5% were 24-year-olds (n=2). In terms of the class year of the respondents, 26.6% were freshmen (n=21), 19% were sophomores (n=15), 31.6% were juniors (n=25), and 22.8% were seniors (n=18). Lastly, for the racial/ethnic makeup of the sample, 76.9% of respondents were White/Caucasian (n=60), 15.4% were Black or African American (n=12), 5.1% were Hispanic, Latino, or of Spanish origin (n=4), 1.3% were Mixed (n=1), and 1.3% were White/Asian (n=1). See Table 1 for the complete breakdown of the demographic descriptive statistics of the sample.

Table 1

Sample Demographic Descriptive Statistics

	N	***%***	***M***	***SD***	***Median***	***Mode***	***Range***
Gender	79		-	-	-	2	3
Male	20	25.3					
Female	58	73.4					
Cisgender	1	1.3					
Age	79		20.09	1.425	20	21	7
18	11	13.9					
19	21	26.6					
20	12	15.2					
21	25	31.6					
22	7	8.9					
23	1	1.3					
24	2	2.5					
Class Year	79		-	-	3	3	4
Freshman (1st Year)	21	26.6					
Sophomore	15	19.0					
Junior	25	31.6					
Senior	18	22.8					
Race/Ethnicity	79		-	-	-	1	5
White/Caucasian	60	76.9					
Black or African American	12	15.4					
Hispanic, Latino, or Spanish	4	5.1					
Mixed	1	1.3					
White/Asian	1	1.3					

Independent Variables: Anxiety and Depression Symptoms

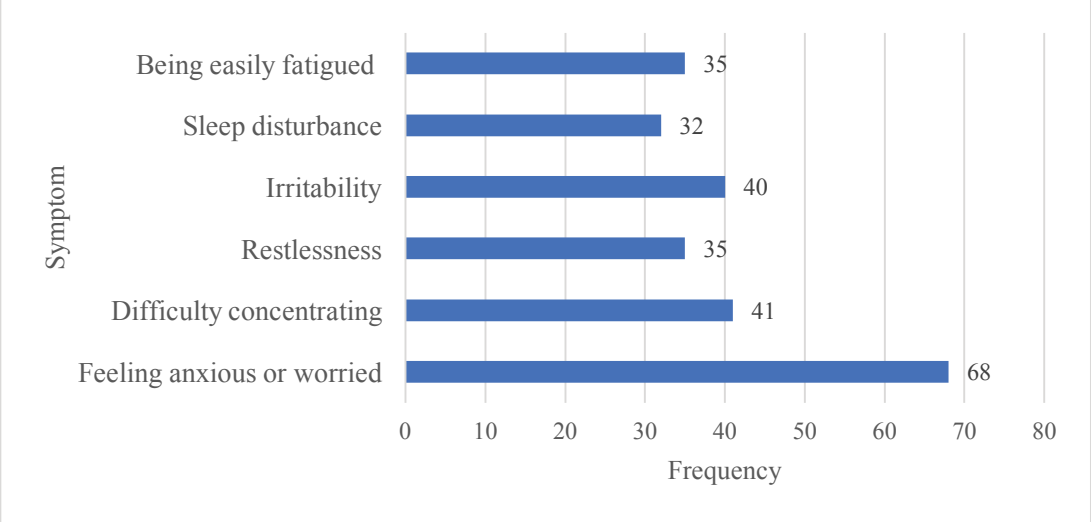
The first independent variable of this study was anxiety. This was measured by asking participants about their symptoms of generalized anxiety, as defined by the DSM-5. The DSM-5 defines anxiety as "Excessive anxiety and worry (apprehensive expectation), occurring more days than not for at least six months, about a number of events or activities" (APA, 2013, p. 222). Participants were asked:

1. Have you ever been diagnosed with any form of anxiety? (1=Yes, 2=No) (Watson et al., 2007). The sample consisted of 61.5% of respondents answering no (n=48) and 38.5% responding with yes (n=30). The mode for this measure was no
2. In the past two weeks, have you felt/experienced any of the following (mark all that apply)? (1=Feeling anxious or worried, 2=Difficulty concentrating, 3=Restlessness, 4=Irritability, 5=Sleep disturbance, 6=Being easily fatigued) (Prevatt et al., 2015). The sample contained several different combinations of symptoms, but feeling anxious or worried was the mode, with 93.2% saying they had felt this in the past two weeks (n=68). See Figure 1 for the distribution of anxiety symptoms.
3. Of the symptoms marked in the previous question, how severe would you consider them to be? (1=Not applicable, 2=Not at all, 3=A little bit, 4=Moderately, 5=Quite a bit, 6=Extremely) (Watson et al., 2007). As for this, 34.2% of respondents said that these symptoms were a little bit severe (n=27), 32.9% said they were moderately severe (n=26), 24.1% said not severe at all (n=19), the question was not applicable to 7.6% of respondents (n=6), and 1.3% said quite a bit severe (n=1). The median and mode for this measure were a little bit severe.

4. In the past two weeks, how frequently have you noticed the symptoms marked above being experienced? (1=Not applicable, 2=Once a week, 3=Twice a week, 4=Every other day, 5=Almost everyday, 6=Everyday) (Watson et al., 2007). For this, 34.2% of respondents said twice a week (n=27), 21.5% said every other day (n=17), 16.5% said almost everyday (n=13), 15.2% said once a week (n=12), 10.1% were not applicable to this question (n=8), and 2.5% said everyday (n=2). The median and mode for this measure were twice a week.

Figure 1

Distribution of Anxiety Symptoms in the Past Two Weeks



The second independent variable in this study is depression. This is defined as "Depressed mood (sad, empty, hopeless) most of the day, nearly every day; markedly diminished interest or pleasure in all, or almost all activities" (APA, 2013, p.160). Participants were asked the following questions. Please see Table 2 for the complete breakdown of the independent variable frequencies and descriptives.

1. Have you ever been diagnosed with any form of depression? (1=Yes, 2=No) (Watson et al., 2007). The sample consisted of 79.5% of respondents answering no (n=62) and 20.5% responding with yes (n=16). The mode for this measure was no.
2. In the past two weeks, have you felt/experienced any of the following (mark all that apply)? (1=Exhausted, 2=Depressed, 3=Inadequate, 4=Fidgety/restless, 5=Had thoughts of suicide, 6=Slept very poorly, 7=Blamed myself for things, 8=Had trouble falling asleep, 9=Felt discouraged about things, 10=Thought about hurting myself, 11=Did not have much of an appetite, 12=Felt like eating less than usual, 13=Had little interest in my usual hobbies or activities, 14=Had trouble concentrating, 15=Had trouble making up my mind, 16=Talked more slowly than usual, 17=Found myself worrying all the time, 18=Took a lot of effort for me to get going) (Watson et al., 2007). The sample contained several different combinations of symptoms, but feeling exhausted was the mode, with 80.6% saying they had felt this in the past two weeks (n=58). See Figure 2 for the distribution of depression symptoms.
3. Of the symptoms marked in the previous questions, how severe would you consider them to be? (1=Not applicable, 2=Not at all, 3=A little bit, 4=Moderately, 5=Quite a bit, 6=Extremely) (Watson et al., 2007). As for this, 32.9% of respondents said that these symptoms were not severe at all (n=26), 32.9% said they were a little bit severe (n=26), 22.8% said moderately severe (n=18), the question was not applicable to 10.1% of respondents (n=8), and 1.3% said quite a bit severe (n=1). The modes for this measure were not severe at all and a little bit severe. The median was a little bit severe.
4. In the past two weeks, how frequently have you noticed the symptoms marked above being experienced? (1=Not applicable, 2=Once a week, 3=Twice a week, 4=Every other

day, 5=Almost everyday, 6=Everyday) (Watson et al., 2007). For this, 34.2% of respondents said twice a week (n=27), 27.8% said once a week (n=22), 20.3% said almost everyday (n=16), 8.9% were not applicable to this question (n=7), 6.3% said almost everyday (n=6), and 2.5% said everyday (n=2). The median and mode for this measure were twice a week.

Figure 2

Distribution of Depression Symptoms in the Past Two Weeks

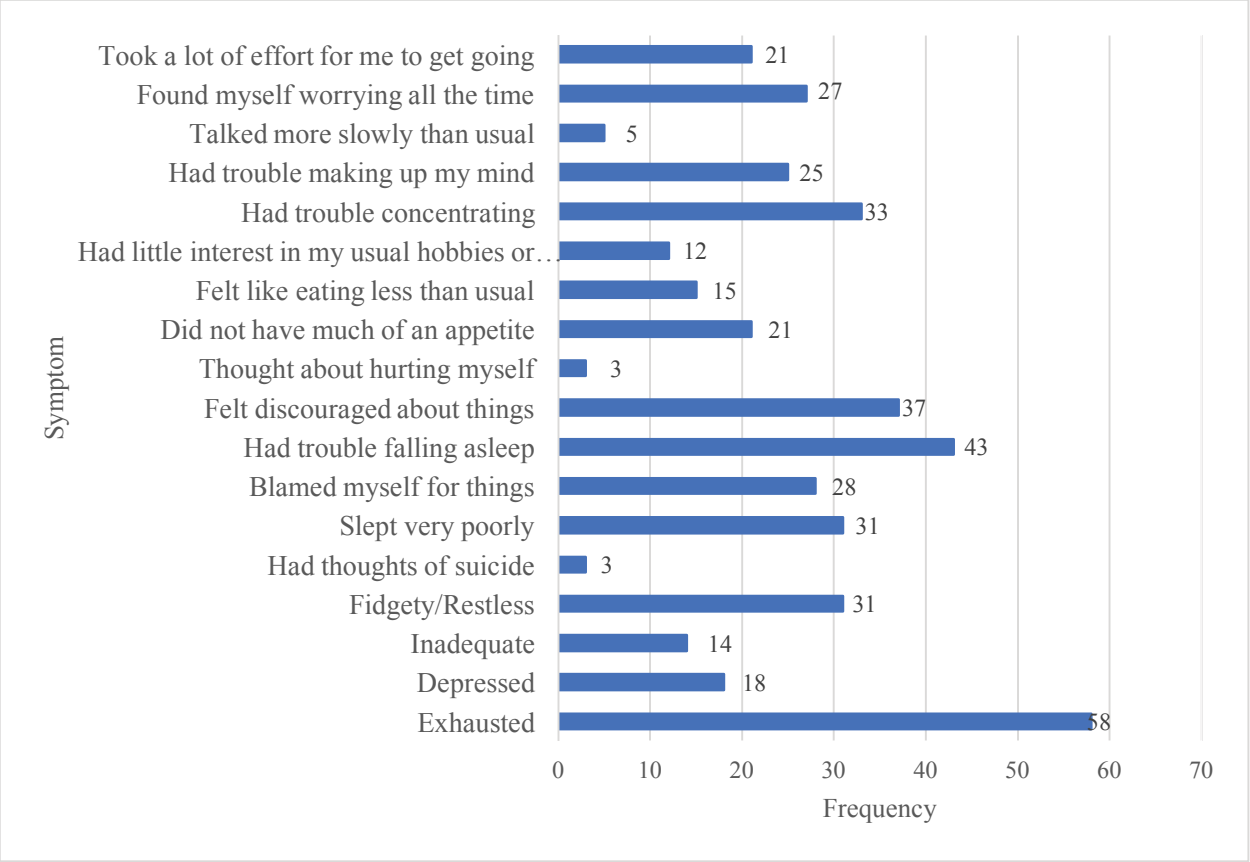


Table 2

Independent Variable Descriptive Statistics

	N	*%*	*M*	*SD*	*Median*	*Mode*	*Range*
Diagnosed Anxiety	78		-	-	-	2	2
Yes	30	38.5					
No	48	61.5					
Anxiety Symptom Severity	79		-	-	3	3	5
Not applicable	6	7.6					
Not severe at all	19	24.1					
A little bit severe	27	34.2					
Moderately severe	26	32.9					
Quite a bit severe	1	1.3					
Anxiety Symptom Frequency	79		-	-	3	3	6
Not applicable	8	10.1					
Once a week	12	15.2					
Twice a week	27	34.2					
Every other day	17	21.5					
Almost everyday	13	16.5					
Everyday	2	2.5					
Diagnosed Depression	78		-	-	-	2	2
Yes	16	20.5					
No	62	79.5					
Depression Symptom Severity	79		-	-	3	2, 3	5
Not applicable	8	10.1					
Not severe at all	26	32.9					
A little bit severe	26	32.9					
Moderately severe	18	22.8					
Quite a bit severe	1	1.3					
Depression Symptom Frequency	79		-	-	3	3	6
Not applicable	7	8.9					
Once a week	22	27.8					
Twice a week	27	34.2					
Every other day	16	20.3					
Almost everyday	5	6.3					
Everyday	2	2.5					

Dependent Variables: Alcohol & Drug Misuse

To measure alcohol use, this study asked about participants' binge drinking habits. Binge drinking is defined by the CDC as four or more drinks consumed in two hours for women, or five or more drinks consumed in two hours for men (CDC, 2019). Respondents were asked:

1. How many times have you participated in binge drinking as it is defined by the CDC, in the past 30 days? (1=0 times, 2=1-2 times, 3=3-4 times, 4=5-6 times, 5=7-8 times, 6=9 times, 7=10 or more times) (University of Delaware, 2017). In the last 30 days, 40.8% of participants responded that they had binge drank 0 times (n=31), 25% said 1-2 times (n=19), 11.8% said 5-6 times (n=9), 10.5% said 3-4 times (n=8), 6.6% said 9 or more times (n=5), and 5.3% said 7-8 times (n=4). The mode for this measure was 0 times, and the median was 1-2 times.
2. About how many times have you participated in binge drinking in the past year? (1=0 times, 2=1-2 times, 3=3-5 times, 4=6-9 times, 5=10-19 times, 6=20-39 times, 7=40 or more times) (University of Delaware, 2017). In the last year, 28.9% of participants responded that they had binge drank 0 times (n=22), 18.4% said 3-5 times (n=14), 14.5% said 20-39 times (n=11), 14.5% said 40 or more times (n=11), 9.2% said 10-19 times (n=7), and 5.3% said 6-9 times (n=4). The mode for this measure was 0 times, and the median was 3-5 times.

Then, in terms of drug misuse, it was defined as the use of any drugs, legal or illegal, not as prescribed by a medical professional (CDC, 2019). This was assessed by asking participants about how often they had misused drugs. Respondents were asked the following questions. Please see Table 3 for the complete breakdown of the dependent variable frequencies and descriptives.

1. How often have you used any of the following: cigarettes, chewing tobacco, snuff, dip, snus, cigars, cigarillos, little cigars, Black and Milds, E-cigarettes, other vaping devices, or hookah? (1=Never, 2=Before but not in the past year, 3=A few times in the past year, 4=Once or twice a month, 5=Once or twice a week, 6=Almost everyday) (University of Delaware, 2017). For this, 47.4% said they had never used these products (n=36), 21.1% said almost everyday (n=16), 15.8% said before but not in the past year (n=12), 10.5% said a few times in the past year (n=8), 3.9% said once or twice a month (n=3), and 1.3% said once or twice a week (n=1). The mode for this measure was never, and the median was before but not in the past year.
2. How often have you used marijuana or cannabis products that are not prescribed by a doctor/medical professional? (1= Never, 2=Before but not in the past year, 3=A few times in the past year, 4=Once or twice a month, 5=Once or twice a week, 6=Almost everyday) (University of Delaware, 2017). For this, 52.6% said they had never used marijuana/cannabis products (n=40), 22.4% said before but not in the past year (n=17), 13.2% said a few times in the past year (n=10), 3.9% said once or twice a month (n=3), 3.9% said once or twice a week (n=3), and 3.9% said almost everyday (n=3). The median and mode for this measure were never.

Table 3

Dependent Variable Descriptive Statistics

	N	*%*	*M*	*SD*	*Median*	*Mode*	*Range*
Binge drinking in the past 30 days	76		-	-	2	1	6
0 times in the last 30 days	31	40.8					
1-2 times in the last 30 days	19	25.0					
3-4 times in the last 30 days	8	10.5					
5-6 times in the last 30 days	9	11.8					
7-8 times in the last 30 days	4	5.3					
9 or more times in the last 30 days	5	6.6					
Binge drinking in the past year	76		-	-	3	1	7
0 times in the past year	22	28.9					
1-2 times in the past year	7	9.2					
3-5 times in the past year	14	18.4					
6-9 times in the past year	4	5.3					
10-19 times in the past year	7	9.2					
20-39 times in the past year	11	14.5					
40 or more times in the past year	11	14.5					
Tobacco product use	76		-	-	2	1	6
Never	36	47.4					
Before but not in the past year	12	15.8					
A few times in the past year	8	10.5					
Once or twice a month	3	3.9					
Once or twice a week	1	1.3					
Almost everyday	16	21.1					
Marijuana use	76		-	-	1	1	6
Never	40	52.6					
Before but not in the past year	17	22.4					
A few times in the past year	10	13.2					
Once or twice a month	3	3.9					
Once or twice a week	3	3.9					
Almost everyday	3	3.9					

Plan for Analysis

Two Spearman correlations were conducted to determine if there are any relationships between the anxiety symptoms and substance use habits of college students, as well as a Spearman correlation used to determine if there were any relationships between the depressive symptoms and substance use habits of college students.

Since all independent and dependent variables were ordinal or binary, Spearman correlations were run. Spearman correlations are conducive to analyzing the relationship between several variables at once, as well as being feasible for ordinal and binary variables. The first Spearman correlation for anxiety symptoms and substance will be used to analyze Hypothesis 1: Students who experience generalized anxiety symptoms are more likely to binge drink or misuse drugs than those with fewer/less severe symptoms. Then, the second Spearman correlation for depressive symptoms and substance use will be used to analyze Hypothesis 2: Students who experience depression symptoms are more likely to binge drink or misuse drugs, than those with fewer/less severe symptoms.

Results

Table 4 shows the results of the Spearman correlation that was conducted to analyze the relationship between anxiety symptoms and substance use habits among the participants. In this Spearman correlation, only one significant relationship was found: there was a weak, positive correlation between the variables anxiety symptom frequency and marijuana use $r_s(77) = .333$, $p < .05$. Those who reported more frequent anxiety symptoms also reported more frequent marijuana use. This significant correlation supported Hypothesis 1, in that those with more frequent anxiety symptoms were more likely to misuse substances, in this case marijuana, than those with less frequent symptoms. These were positively correlated, which means that as anxiety symptom frequency increases, so does marijuana use. Approximately 11.08% of the variance is explained by marijuana use. The rest of the variables in the Spearman correlation for anxiety symptoms and substance use were found to be not significant, meaning there was no meaningful relationship between these variables within the sample collected. Since one of the

relationships was significant, but not all, Hypothesis 1 is partially supported by the data collected. See Table 4 for a complete breakdown.

Table 4

Spearman Correlation Anxiety Symptoms and Substance Use

Variables	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*
1) Diagnosed Anxiety	1	-	-	-	-	-	-
2) Anxiety Symptom Severity	-.229	1	-	-	-	-	-
3) Anxiety Symptom Frequency	-.375	.716	1	-	-	-	-
4) Binge Drinking Past Month	.102	-.098	-.055	1	-	-	-
5) Binge Drinking Past Year	.147	-.001	.036	.919	1	-	-
6) Tobacco Product Use	-.123	.213	.187	.175	.234	1	-
7) Marijuana Use	-.175	.186	.333**	.152	.192	.402	1

**$p<.01$

Table 5 shows the results of the Spearman correlation that was conducted in order to analyze the relationship between depressive symptoms and substance use habits among the participants. This table shows that none of the variables were significantly correlated, meaning there were no meaningful relationships found between the depressive symptom variables and the substance use habits of the individuals in this sample. Because of this, Hypothesis 2 was not supported. See Table 5 for a complete breakdown.

Table 5

Spearman Correlation Depressive Symptoms and Substance Use

Variables	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*
1) Diagnosed Depression	1	-	-	-	-	-	-
2) Depression Symptom Severity	.094	1	-	-	-	-	-
3) Depression Symptom Frequency	.127	.000	1	-	-	-	-
4) Binge Drinking Past Month	.347	.086	.359	1	-	-	-
5) Binge Drinking Past Year	.704	.335	.851	.000	1	-	-
6) Tobacco Product Use	.087	.225	.244	.131	.042	1	-
7) Marijuana Use	.074	.126	.158	.189	.097	.000	1

Discussion

The purpose of this study was to examine the relationship between anxiety and depression symptoms with the substance use habits of undergraduate students. The results found in the Spearman correlation between anxiety symptoms and substance use partially supported Hypothesis 1. The only significant relationship was between anxiety symptom frequency and marijuana use, which shows that those who reported more frequent anxiety symptoms were more likely to use marijuana than those with less frequent anxiety symptoms. This was a weak, positive correlation, meaning that when anxiety symptom frequency increases, so does marijuana

use, and vice versa. This significant finding suggests that there may be a relationship between anxiety symptoms and substance use; however, this relationship was specifically found in marijuana use rather than binge drinking or any other drugs that were asked about in the survey.

These findings further support claims by Bravo & Pearson (2017) that college students gain several newfound freedoms, and many students struggle with these new responsibilities, so they use alcohol and drugs as a way to cope with the new lifestyle. Since marijuana has depressant properties, it is often used as a way to relax, which students may use to combat their overwhelming anxiety symptoms (Saini & Suthar, 2022). Though alcohol is a depressant as well, alcohol is known to be more of a social substance that is consumed in larger groups, as opposed to marijuana, which is more commonly used by oneself or in a smaller group (Bravo & Pearson, 2017). This might explain why there was a correlation between marijuana and anxiety, but not alcohol and anxiety. If someone is feeling anxious, they may turn to marijuana as a remedy for their anxiety symptoms, whereas alcohol is more often seen as a substance that is more often consumed in social settings, and less commonly used to combat anxiety symptoms. The results from this study confirm these ideas as well since there is a significant relationship between anxiety symptom frequency and marijuana use.

As for depressive symptoms and substance use, no significant relationships were found. The second Spearman correlation that tested these variables showed no significant relationships. This means that Hypothesis 2 was not supported at all by the data from this sample.

A possible explanation for this is the symptoms that depression brings on. Per the DSM-5, depression is feeling a "Depressed mood (sad, empty, hopeless) most of the day, nearly every day; markedly diminished interest or pleasure in all, or almost all activities" (APA, 2013, p.160). Watson et al. (2007) also note some common symptoms of depression, some of which include:

feeling inadequate, difficulty sleeping, feeling discouraged, not having much of an appetite, and having little interest in usual hobbies or activities. Because these symptoms cause people to be tired or lose interest in activities, this may be a reason people are not inclined to drink or use drugs when depressed (Wang et al., 2018). As opposed to anxiety, depression often makes the sufferer feel like they have little will to live or do anything, which could be a reason there is no significant relationship between depressive symptoms and substance use. Sufferers of depression often have so little motivation that they may not even feel like drugs or alcohol will help (Wang et al., 2018). This is likely why none of the depressive variables had a significant relationship with the substance use habits of respondents.

Limitations

Despite efforts to maintain the validity and accuracy of this study, some major limitations still exist. The biggest limitation is the sample size. The study used a nonprobability convenience sampling method and only received 79 responses. Since the sample size was small in terms of a quantitative study, the results may not be very generalizable to the target population as a whole. American society is too diverse to consider a small sample size to be representative of the entire population (Schutt, & Bachman, 2011). Around the country, there are millions of undergraduate students, all with different backgrounds and life experiences (Kraft et al., 2021). Because of this, it is ignorant to say that the 79 participants in this study could be representative of all undergraduates around the nation.

Secondly, an availability sampling method also poses a threat to the study's validity. Availability samples are beneficial because of their convenience, hence why they are often called convenience samples, making it easier to get participants. Survey participants are gathered based on their availability (Schutt, & Bachman, 2011). In this case, respondents were recruited through

emails from professors and text messages from researchers with the survey link. This means that anyone with the survey link could respond, but since it is voluntary, no one technically had to complete the survey. This gives the researchers little to no control over the respondents and who is included in the sample (Schutt, & Bachman, 2011). Since availability sampling was conducted, the study is not generalizable to the larger population of undergraduate students at Cabrini University.

Next, the demographics of this study were representative of the demographics at Cabrini University; however, this is one small-sized university in the suburbs of Philadelphia. Cabrini is a predominantly white female student population. It is also a Catholic and liberal arts school. All these variables can play a role in respondents' answers to the survey. Gathering data from one small university makes it difficult to get demographics that are representative of the national undergraduate population. Because of this, the results are not as generalizable as they would be if data was gathered from more universities. This would allow for more diversity in the sample demographics and participant backgrounds, which would make it more representative of the population of all undergraduate students in the United States.

Another limitation in this study is temporal ordering. Because the study was not longitudinal, there was no way to know which variable occurred first in the students' lives. In this study, anxiety and depression symptoms are independent variables; however, there was no control to determine whether these were present before asking about the substance use habits of participants. Someone could have had anxiety or depression symptoms, which made them more likely to use substances, or they could have used substances that perpetuated their anxiety or depression symptoms. This limitation in the study makes it difficult to tell which variables come first and what the actual cause of substance use might be.

Implications

Overall, this study suggests that there is a potential relationship between anxiety symptoms and substance use in undergraduate students. With the significant relationship between anxiety symptom frequency and marijuana use, future research could investigate why these variables might be correlated. Researchers could at why this relationship exists and why, specifically, marijuana is often used to combat anxiety symptoms as opposed to other substances, like alcohol and other drugs.

As a whole, this research is very pressing not only for college students but everyone. Since everyone has a stake in the higher education system in some way, it is important that everyone is concerned about the mental health and well-being of today's undergraduate population. College students could use this information to gain more insight into their substance use habits; university staff could use this to improve policies and programs provided to undergraduate students; legislators could use this to better inform the policies they make surrounding substance use, specifically in college-aged people; researchers could use this as background to further explore the relationships between mental health and college students. Because of the versatility of this study, it is incredibly important and useful in the field of social science.

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Investigating the role of the major capsid protein as a biomarker for horizontal gene transfer between bacteria and bacteriophages

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Abstract

Horizontal gene transfer (HGT) plays a beneficial role in the evolution and survival of bacteriophages and bacteria. The extent of HGT between *Streptococcus* bacteria and associated bacteriophages, focusing on viral major capsid proteins, was studied utilizing a bioinformatics approach. Evidence of HGT was identified via the community science analysis pipeline and the BLAST database. Evolutionary relationships were assessed using MEGA software to construct phylogenetic trees. Overall relationships were then represented as networks via the Gephi application. Literature has shown that the major capsid protein in bacteria works analogously to bacterial microcompartments, protecting genetic materials and organelles. These observations, as well as genomic locations of genes coding for major capsid proteins, DNA polymerases, DNA topoisomerases, and other associated molecules, have led to their uses as biomarkers of potential HGT cases. The results provide evidence of extensive HGT between bacteria and bacteriophages, which helps in understanding their evolution and potential therapeutic uses.

Introduction

Antibiotic resistance within bacterial populations is rising to dangerously high levels and new resistance mechanisms are emerging and spreading globally. One such mechanism that has been observed is horizontal gene transfer (HGT). HGT occurs when genetic material is exchanged between organisms in a non-genealogical manner (Goldenfeld and Woese, 2007). This genetic exchange is unlike the genetic exchange which occurs from parent to offspring, as HGT usually occurs between different organisms which are not related. Through the means of HGT, a bacteria can pick up many different functions, including antibiotic resistance and virulence factors. (Deng et al. 2019)

Bacteriophages have shown to have a role in transfer of genetic material between bacteria (Borodovich et al., 2018). Bacteriophages can infect bacteria either through the lytic cycle (called lytic phages) or the lysogenic cycle (called temperate phages) (Rehman et al., 2019). The lysogenic cycle involves the bacteriophage integrating their genome into that of the host cell, and can become dormant, only to infect the cell when it undergoes activation (Labonté et al., 2019). These temperate phages can serve as vectors for HGT between bacterial species via transduction (Labonté et al., 2019). Phage transduction can be studied by examining a bacterial genome and

locating the pockets of viral DNA. Phages have effects on the control of bacterial populations, the spread of virulence factors and antibiotic resistance genes, resulting from unique combinations of genetic diversity (Cumby et al., 2015). The mechanism by which these viruses infect bacteria and how these drive their evolution is poorly understood and is crucial to understand where they originated from (Cumby et al., 2015). Bacteriophages have unique host range, and their specificity is determined by their specific structures to attach to specified host bacterial cell receptors and infect the cells.

The protein that we focus on in our study is the major capsid protein. Capsids are the morphological structures that contain the condensed form of the genetic material of a bacteriophage, and also protect it from any outside physical and chemical damages. Recent research has shown that mutations in the genes that code for these proteins are necessary for certain interactions with different host cell receptors and appear to contribute to the stability of a given capsid. This suggests that these mutations aid in broadening bacteriophages' host ranges (Labrie et al., 2014).

Interestingly, there are similarities between encapsulin proteins that form bacterial structures resembling shells, and the major capsid protein of the HK-97 bacteriophage (Freire et al., 2015). Structural identities are also seen with capsid proteins and S-layer lattice protein components of the cell envelope of prokaryotes and bacteria (Freire et al., 2015). Fusogenic proteins of enveloped viruses which enable the fusion between them and host cell membranes, have also been shown to function analogously to the SNARE family of proteins of *Caenorhabditis elegans*, which encode for fusion of intracellular vesicles to their cell membranes and allow for cell–to–cell communication (Freire et al., 2015). Previous studies have found similar structures and functions of major capsid proteins and bacterial microcompartments.

Bacterial microcompartments are protein shells that encase enzymes, molecules essential for the microorganism, and may be even their genetic material, protecting these items from degradation or physical or chemical damages in or out of the cell (Krupovic & Koonin, 2017). Similarities between bacterial structures and capsid proteins provide insight into how the proteins encoding capsids have expanded from beyond bacteriophages.

Bioinformatics analyses and studies have demonstrated bacteria that have undergone HGT with bacteriophages possess conserved genomic regions pertaining to not just to single genes, but multiple, different genes that are located relatively close to one another. According to research presented by Sabath et al., 2012, overlapping genes and sequences are very common in viral genomes. Expressions of these genes have been confirmed, while functionality requires further investigation. Interestingly, the resulting, translated proteins lack a stable, tertiary, three-dimensional structure characteristic of most normal, wild-type proteins (Sabath et al., 2012). In addition to genes encoding major capsid proteins, genes that encode for DNA polymerases, DNA topoisomerases, and other molecules associated with genome replication are inherited through HGT as well. These genes have been shown to be located close together within viral genomes. Appropriately, these genes are termed genomic islands, which refer to groups of unique open reading frames that contain sequences that encode given traits or carry out specific functions (Villa & Viñas, 2019). Examples of genomic islands that have been investigated carry out functions pertaining to virulence and pathogenicity, symbiosis, metabolism, fitness, and antibiotic resistance (Finke et al., 2017). The specific mechanism of HGT involved in how these genomic islands are integrated into the genomes of host organisms remains unknown (Villa & Viñas, 2019). This property provides an opportunity to utilize major capsid proteins as

biomarkers when analyzing genomic sequences to establish evidence of HGT amongst bacteriophages and bacteria (Born et al., 2019).

In this study we focused on the major capsid protein in *Streptococcus genus of* bacteria. Studying how bacteria acquires its resistance to antibiotics is necessary, due to the shrinking list of effective antibiotics. The objective of this study is to assess the extent of HGT between bacteria species and associated bacteriophages, using the major capsid proteins as biomarkers. Our results indicate that overlapping open reading frames composed of varying numbers of base pairs are located close to the major capsid protein in the bacterial genomes. Whether or not these regions encode functional proteins is not entirely known. Current annotations present in databases indicate that these genomic regions encode several different proteins that contribute to the structure and morphology of the major capsid protein (Rosenwald et al., 2014).

Methods

HGT & Community Science Project Pipeline

Positive cases of HGT between bacteriophages and bacteria were determined using the Community Science Project Pipeline (Mathur et al., 2019). A list of accession numbers of bacteriophage major capsid proteins was generated from the NCBI database (Sayers et al., 2022). Each phage accession number was searched against the bacteria database on NCBI using BLASTp to generate positive hits (referred to as Forward BLAST) (Johnson et al., 2008). The top 10 hits based on the cut-off criteria of e-values of 1e-50 or lower, and a query coverage of 70% or higher, were recorded. The top bacterial hit accession number was then searched against the Virus database on NCBI using BLASTp (referred to as Reverse BLAST) (Johnson et al., 2008). Again, the top 10 hits which satisfied the cut-off parameters were recorded. If the top virus hit accession number in the Reverse BLAST matched the original virus accession number

query, that bacteria-virus pair was recorded as a potential positive case of HGT. In total, 75 phage accession numbers were tested to give 21 positive HGT bacteria-virus pairs (**Table 1)**.

Evolutionary History of Bacteria and Bacteriophages - Comparative Genomics

The evolutionary history of bacteria and bacteriophages was assessed via comparative genomics. FASTA sequences of the major capsid protein from all positive cases of HGT were uploaded to MUSCLE software and aligned (Edgar, 2004). **(Supplementary Figure 1)**. These sequences were then uploaded to the MEGA7 software to generate phylogenetic trees (Kumar et al., 2015). The phylogenetic tree was constructed based on maximum likelihood method and bootstrapping value of 100. Based on the results, the *Streptococcus* clade of bacteria was selected for further analyses.

Synteny & Evolutionary Relationships

The *Streptococcus* clade of bacteria and bacteriophages were selected for the synteny analysis. Synteny for the *Streptococcus* clade of bacteria and bacteriophages was determined using the software MAUVE (Darling et al., 2010). Major capsid gene sequences were downloaded from NCBI for both bacteria and bacteriophages. The Mauve synteny output was generated for all the phages, bacteria and the visualization of the bacteria and phage sequence.

Gephi Network Analysis

The top six results from the Forward and Reverse BLAST searches were collected based on the Community Science Pipeline and were each organized as a node into the Gephi software for network analysis (Bastian et al., 2009). Connections between bacteria and bacteriophages based on the generated phylogenetic trees were input into Gephi as edges. A node in the center of the network with the most edges connected to it was indicative of the ancestral sequence that was

shared by most bacteria and bacteriophages through HGT.

Results

Comparative Genomics

Based on the arrangement on the phylogenetic tree (Figure 1.) and validation by the bootstrapping values, there is a high likelihood that *Streptococcus* bacteriophages and bacteria were involved in HGT with respect to the major capsid protein. Despite the major capsid protein being present in the five bacteriophages and bacteria, their location in the genomes of each species varies. This suggests mutations such as translocations and insertions have occurred over time (Kyrillos, et al., 2016). This could explain the divergence of pairs of bacteriophages and bacteria in the phylogenetic tree. One pair that is the most divergent and in its own unique clade and not associated with the other pairs is the connection between the *Streptococcus* phage VS-2018a and the major capsid protein E in *Streptococcus thermophilus*. This is also reflected in their MUSCLE alignments that vary compared to the other bacteria and bacteriophage pairs.

Synteny & Evolutionary Relationships

The Mauve software was used to create a multiple sequence alignment and predict synteny of Javan *Streptococcus* bacteriophage and bacteria pairs using the progressive Mauve algorithm. In the synteny map of the four Javan prefixed bacteriophages, the major capsid protein lies in the range of approximately 500-2000 base pairs. (**Supplementary Figure 3)**. There is a consistent alignment based on the peak height and coloration patterns with Phage VS2018 having the most unique genome arrangement. *S.thermophilus* is missing a 400 base pair region upstream of the major capsid protein gene, as indicated by a shift in the sequence alignment (**Supplementary Figure 4)**. The five phage sequences of interest are in reverse orientation in the genome indicated by the peaks falling below the main sequence line in **Figure**

2. The area between 850-970 base pairs is a unique region found only in *S.thermophilus* bacteria and the phage VS 2018a pair. This is expected as this pair lies on a separate clade in the phylogenetic tree generated previously. The alignment of the genomes indicates that the region upstream and downstream of the major capsid gene is also shared between these bacteria and bacteriophage pairs. This pattern indicates that there is not just the major capsid gene that is shared between bacteria and phages but instead a whole chunk of the genome.

Gephi Network Analysis

The central node in the network corresponds to a hypothetical protein in *Streptoccoccus pyogenes*. As seen in the top six results of the Reverse BLAST, this node has multiple shared edges with a major capsid protein in *Streptococcus* bacteriophages Javan 146, 454, 464, 474, 459, 484, 166 (**Figure 3)**. The generated network shows that the connections are the same as they appear in the phylogenetic tree. The central node of the gene encoding a hypothetical protein in *S.pyogenes* connects closely to different strains of itself and a Javan bacteriophage 464. This relationship suggests that those two bacteria and bacteriophage pairs could be where the initial transfer of genomic material had occurred.

Discussion

HGT of the major capsid protein has allowed for *Streptococcus* bacteriophages and bacteria to survive in constantly changing environments. HGT increases genetic diversity which may increases development of new adaptations and overall evolution. As suggested by Krupovic & Koonin (2017), the major capsid proteins could serve as a bacterial microcompartment protecting the bacterial genome from physical and chemical damages akin to the functions of viral capsids.

It has been suggested that bacterial genes acquired through HGT are usually quickly deleted from the genome unless they are to be utilized for some specific reasons later (Rosenwald et al., 2014). For example, genes acquired through HGT that improve metabolism in bacteria can be expressed under given circumstances. It is upon changes in the environment or medium that render these genes functionless that can result in the deletion of the genes, as bacterial genomes tend to be compact and constituent (Moran, 2002). Gene deletion most likely occurs to conserve internal energy by not expressing unnecessary genes. Future studies can determine gene deletion frequency via RNA sequencing techniques in which the protein of interest is isolated, and its messenger RNA is extracted and purified from other RNA molecules (Rosenwald et al., 2014).

By identifying HGT within the major capsid protein sequences for the *Streptococcus* bacteria and the bacteriophages that infect them, we can begin to understand the extent of HGT within bacterial populations. We propose that the major capsid protein can be used as a biomarker to identify HGT in other bacteria species as well. There is evidence to suggest that when genes are transferred horizontally, it is not just a single gene but a whole genomic region consisting of multiple genes (Szöllősi et al., 2015). A future direction of this research would be to identify the gene regions flanking the major capsid protein in the bacterial genome and understand the functionality of those genes and the role they play within the bacteria.

In this study, we focused on the *Streptococcus* genus, but it can easily be expanded to include a larger dataset of bacteria and bacteriophage pairs based on data availability in the NCBI database. It is imperative to study the extent and rate of HGT in bacterial populations as it is a key mechanism for bacteria to acquire antibiotic resistance genes, and thus has implications for human health worldwide.

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Table 1: Table of the positive cases of HGT amongst pairs of bacteriophages and bacteria.

Bacteriophage	Bacteriophage Accession Number	Bacteria	Bacteria Accession Number
putative head protein [Riemerella phage RAP44]	YP_007003622.1	hypothetical protein [Riemerella anatipestifer]	WP_014938289.1
putative head protein [Brevibacillus phage Osiris]	YP_009215022.1	hypothetical protein [Brevibacillus laterosporus]	WP_022583694.1)
major capsid protein [Streptococcus phage Javan464]	QBX28740.1	major capsid protein E [Streptococcus pyogenes]	WP_136111941.1
major capsid protein [Arthrobacter phage Isolde] (not top hit)	AYR00888.1	hypothetical protein [Arthrobacter sp. cf158]	WP_091323596.1
phage major capsid protein [Cellulophaga phage phi18:1]	YP_008240963.1	phage major capsid protein [Elizabethkingia anophelis]	WP_059330774.1
phage major head protein [Oenococcus phage phiS13]	YP_009005240.1	hypothetical protein [Oenococcus oeni]	WP_032811398.1
major capsid protein [Streptococcus phage Javan446]	QBX28239.1	hypothetical protein [Streptococcus pyogenes]	WP_014635509.1
major capsid protein [Streptococcus phage Javan146] (not top hit)	QBX23717.1	major capsid protein E [Streptococcus pyogenes]	WP_136022800.1
major capsid protein [Brevibacillus phage Jimmer1]	YP_009226318.1	phage capsid protein [Brevibacillus laterosporus]	WP_119733365.1
major capsid protein [Streptococcus phage Javan166]	QBX23895.1	hypothetical protein [Streptococcus dysgalactiae]	WP_046177708.1
hypothetical protein [uncultured Mediterranean phage uvMED]	BAQ84158.1	hypothetical protein [Elizabethkingia anophelis]	WP_151449511.1
major capsid protein [Streptococcus phage VS-2018a]	AZA24404.1	major capsid protein E [Streptococcus thermophilus]	AZA18259.1

major capsid protein [Streptococcus phage Dp-1]	YP_004306931.1	hypothetical protein D8H99_54145 [Streptococcus sp.]	RKV76237.1
major capsid protein [Mycobacterium phage Renaud18] (not top hit)	AXQ64918.1	MULTISPECIES: major capsid protein E [Mycobacteroides]	WP_057970215.1
prophage major head protein [Oenococcus phage phiS11]	YP_009006573.1	hypothetical protein [Oenococcus oeni]	WP_032811892.1
hypothetical protein [Oenococcus phage phi9805]	YP_009005184.1	hypothetical protein [Oenococcus oeni]	WP_032820248.1
capsid protein [Mycobacterium phage TChen] (not top hit)	AWH14408.1	MULTISPECIES: major capsid protein E [Mycobacteroides]	WP_057970215.1
capsid protein [Arthrobacter phage KellEzio]	YP_009301281.1	hypothetical protein DRJ50_09715 [Actinobacteria bacterium]	RLE21106.1
major capsid protein [Microviridae sp.]	AXH73898.1	hypothetical protein [Elizabethkingia anophelis]	WP_080670996.1
major capsid protein [Streptococcus phage Javan464]	QBX28740.1	major capsid protein E [Streptococcus pyogenes]	WP_136111941.1
major capsid protein [Microviridae sp.]	AXH77365.1		

Figure 1: Phylogenetic tree of all of the positive cases of HGT. There are multiple, unique clades observed. The center *Streptococcus* clade was chosen for further analysis based on the high bootstrap values.

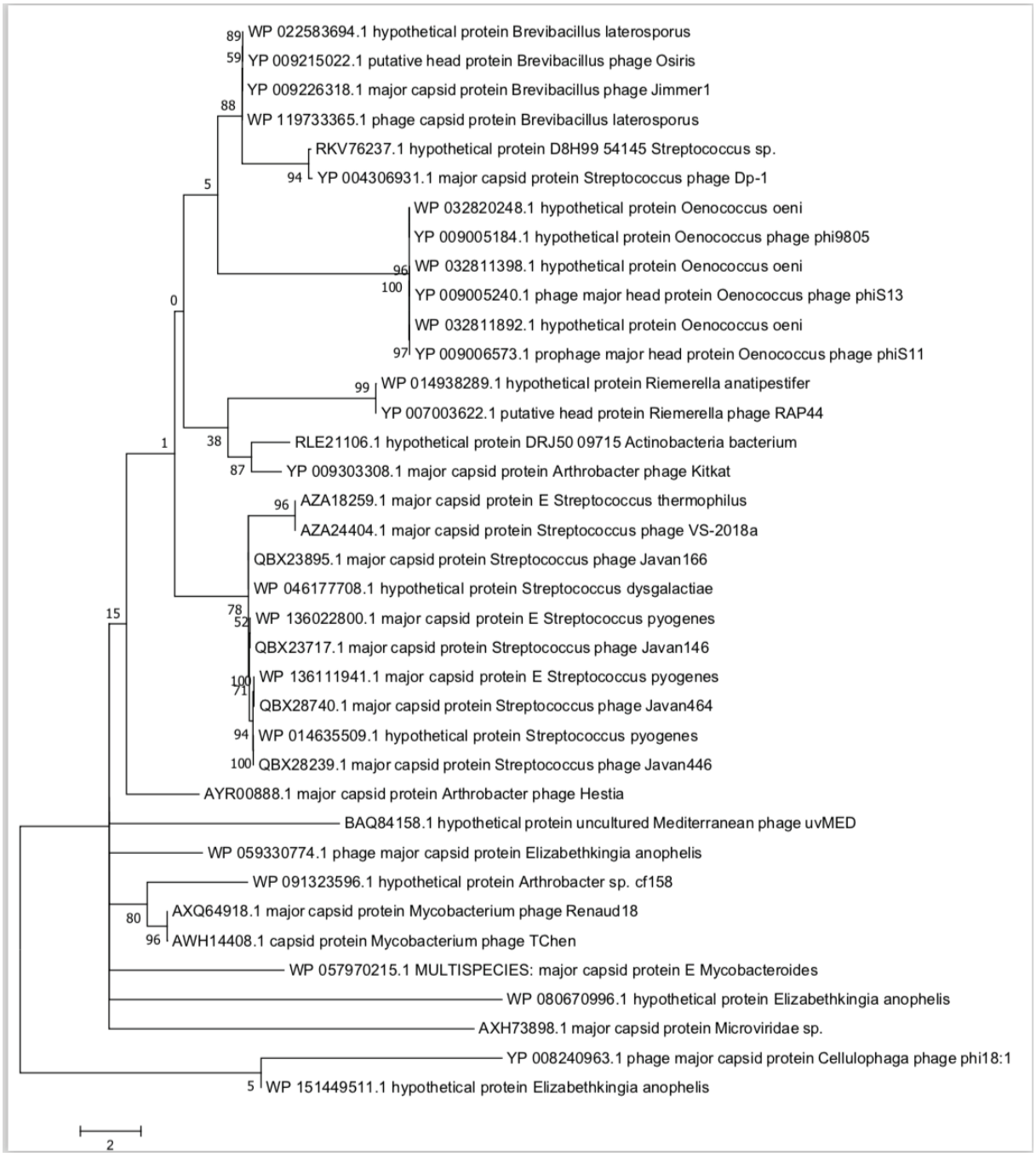


Figure 2: The synteny of the phage and bacteria sequences of interest generated via Mauve. The five phage sequences are in reverse orientation in the genome indicated by the peaks falling below the line. The area between 850-970 base pairs is a unique region that is only found in *S.thermophilus* bacteria and phage VS 2018a pair. This is expected as this pair lies on a separate clade in the phylogenetic tree generated from MEGA7.

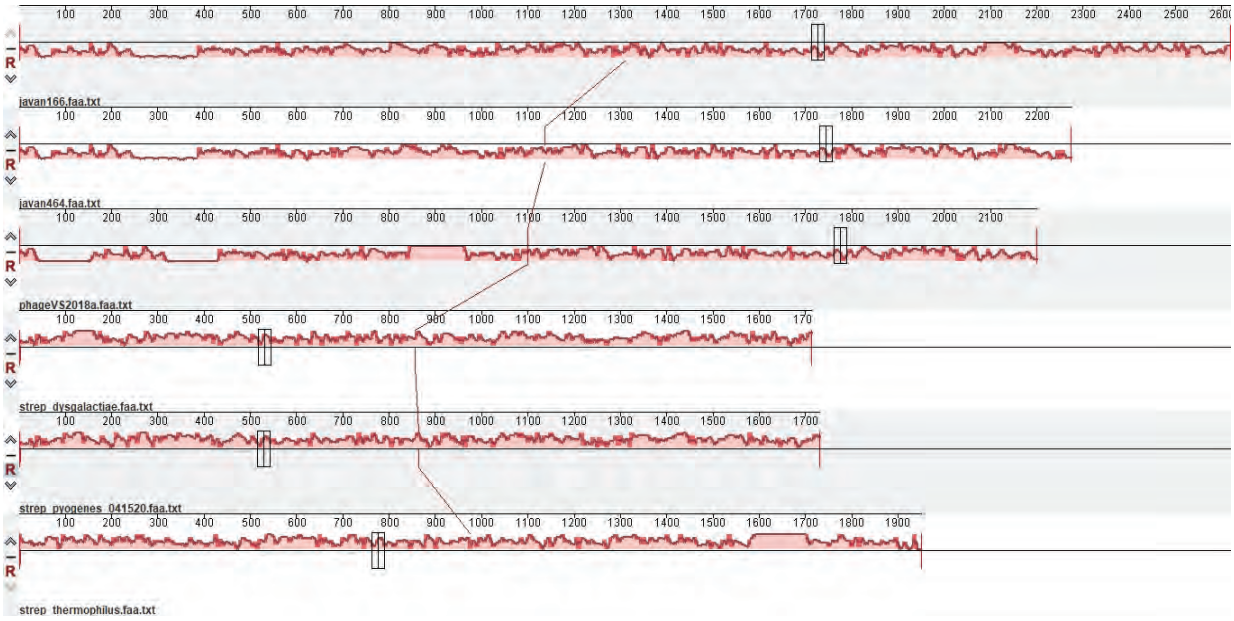
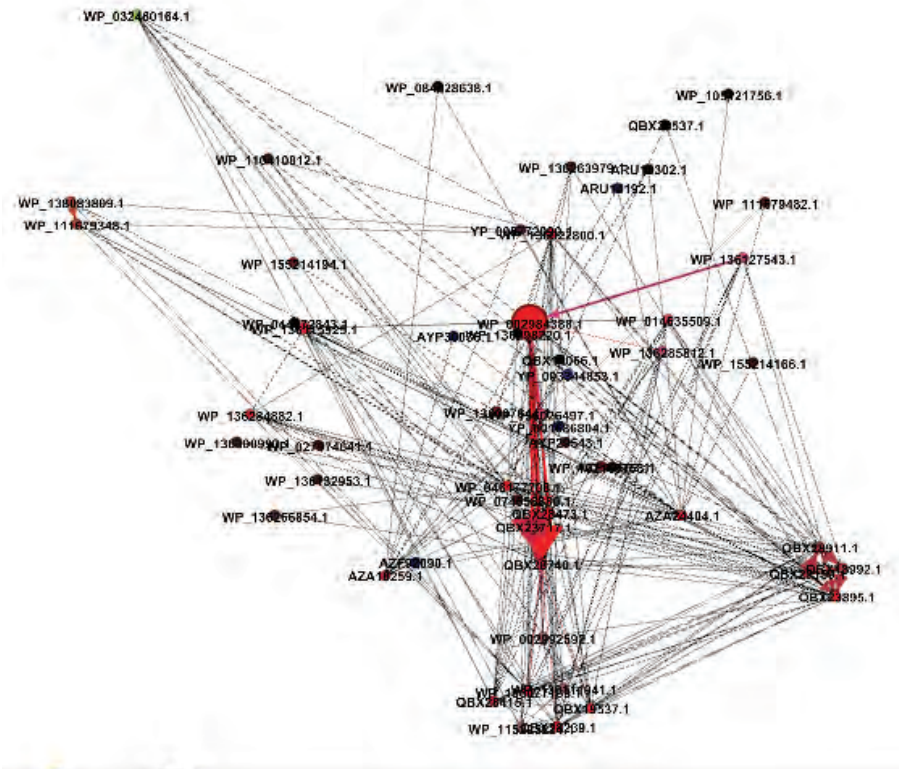


Figure 3: The Gephi network of all positive HGT cases within the *Streptococcus* clade. Notice that all bacteria and bacteriophages display evolutionary relationships through a mechanism of HGT.



Supplementary Figure 1: MUSCLE alignment of the *Streptoccoccus* bacteria and associated bacteriophage pairs. The accession numbers, AZA24404.1, QBX28740.1, QBX28239.1, QBX23895.1, and QBX23717.1 are bacteriophage major capsid proteins. The rest of the sequences are bacterial proteins. All of the sequences are highly conserved here.

```
AZA18259.1          MGLIYDKVTASNIAGYFNALQENVDSTLGESIFPARKQLGTKLSYIKGASGQSVALKAAA
AZA24404.1          MGLIYDKVTASNIAGYFNALQENVDSTLGESIFPARKQLGTKLSYIKGASGQSVALKAAA
WP_136111941.1      MAYIHEIITSENIKGFYDKKKGEVELTLGEKAFPSKQQLGLKLSFIKGASGRPVTLKAAA
QBX28740.1          MAYIHEIITSENIKGFYDKKKGEVELTLGEKAFPSKQQLGLKLSFIKGASGRPVTLKAAA
WP_014635509.1      MAYIHEIITSENIKGFYDKKKGEVELTLGEKAFPSKQQLGLKLSFIKGAAGRPVTLKAAA
QBX28239.1          MAYIHEIITSENIKGFYDKKKGEVELTLGEKAFPSKQQLGLKLSFIKGAAGRPVTLKAAA
WP_046177708.1      MALIHEIITSENIKGFYNAKNENVENTLGENAFPPKQQLGLKLSFIKGAAGKPVTLKAAA
QBX23895.1          MALIHEIITSENIKGFYNAKNENVENTLGENAFPPKQQLGLKLSFIKGAAGKPVTLKAAA
WP_136022800.1      MAYIHEIITSENIKGFYNAKNENVENTLGEKAFPSKQQLGLKLSFIKGAAGKPVTLKAAA
QBX23717.1          MALIHEIITSENIKGFYNAKNENVGNTLGENAFPPKQQLGLKLSFIKGAAGKPVTLKAAA
                    *. *:: :*:.** *:::  : :*  ****. **..:*** ***:****:*..*:*****
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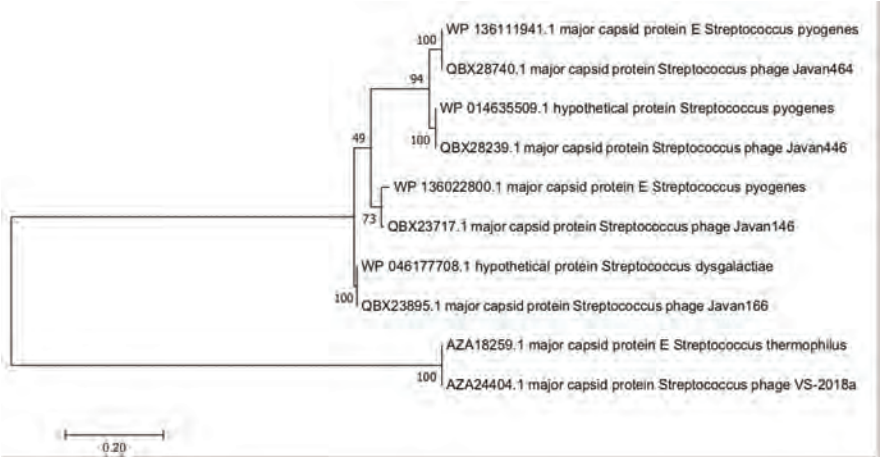
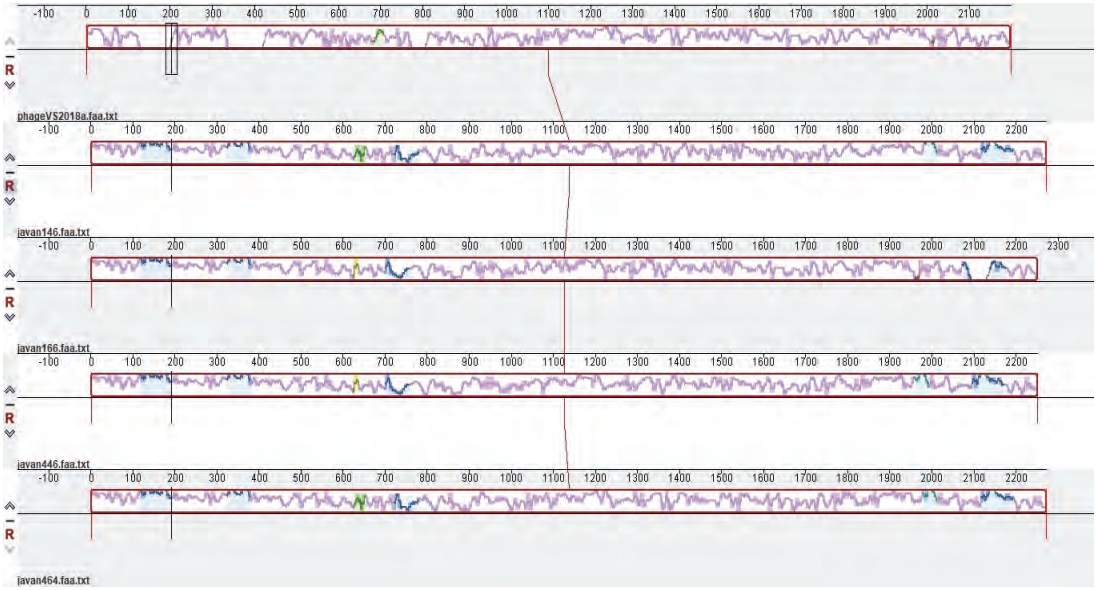
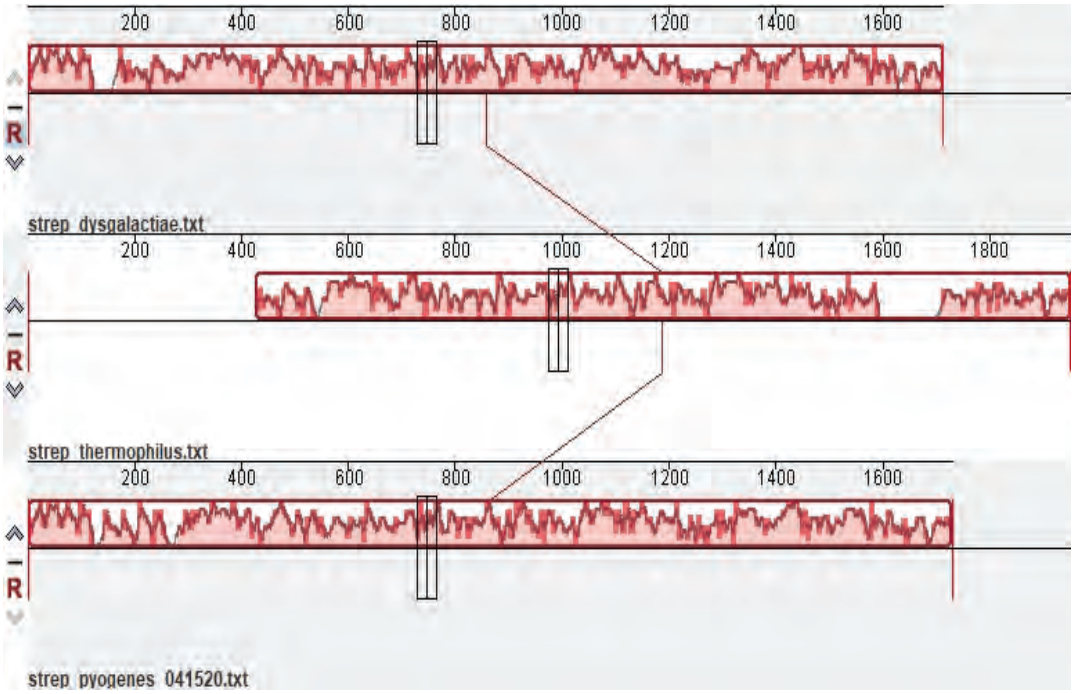
Supplementary Figure 2: Maximum likelihood phylogenetic tree using MEGA7 showing the relationships amongst *Streptococcus* bacteriophages and bacteria.



Supplementary Figure 3: The synteny of the four Javan prefixed phages generated via Mauve. The solid red line connecting each sequence shows the location of the matching section of the genome. The major capsid protein lies in the range of approximately 500-2000 base pairs in this alignment. There is a mostly consistent alignment based on the peak height and coloration patterns. Phage VS2018 has certain regions which are unique as can be seen by the sliding black box feature.



Supplementary Figure 4: The synteny of *S.thermophilus* and *S.dysgalactiae* generated via Mauve. *S.thermophilus* is missing a 400 base pair region upstream of the major capsid protein gene, indicating by the shift in sequence alignment.



Molecular characterization of a class 1 integron *(intl1)* gene in three *Pseudomonas* bacterial species

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Abstract

Pseudomonas bacterial species are aerobic, non-spore forming gram negative bacteria that are characterized as human pathogens and show a high level of antibiotic resistance. Research has shown that there are several ways that bacteria acquire antibiotic resistance including transposons, bacteriophages, plasmids and integrons. For our study, we focused on characterizing a Class 1 integron gene (*intl1*) in three closely related species of *Pseudomonas* bacteria, namely *P. aeruginosa*, *P stutzeri* and *P. putida*. All *intl1* gene sequences found in the three species were downloaded from the NCBI database. We performed a phylogenetic analysis to analyze the similarity between the gene sequences between the three species. Based on the synteny analysis results, we grouped the gene sequences into three distinct categories and compared the results back to the evolutionary relatedness inferred from the phylogenetic tree. The pattern of inheritance indicates a role of lateral gene transfer in the intl1 gene sequences for the bacterial species under study. The class 1 integrons in *Pseudomonas* are associated with high levels of antibiotic resistance in these bacterial species and are also a proxy for environmental health. This study provides insights into the inheritance patterns of *intl1* gene within *Pseudomonas* bacteria and its implications on human health.

Introduction

Integrons are a significant factor in the spread of antibiotic resistance genes in bacteria (Partridge et al., 2001). These genetic elements are mobile and can capture, integrate, and express gene cassettes that encode resistance determinants (Stokes & Hall, 1989). Class 1 integrons are prevalent in clinical isolates of bacteria and are often associated with multidrug-resistant strains (Partridge et al., 2001). Integrons have three specific features which are known as integron-integrase gene (intI), a recombination site (attI) and a promoter (Pc) (Gillings et al., 2015). The purpose of these sites essentially is to capture expressions of exogenous parts of gene cassettes which are then moved to the attI site using intI and expressed from Pc. These integrons are known to move by lateral DNA transfer and contain a wide range of antibiotic resistance (Gillings et al., 2015).

The *intl1* gene is a key component of class 1 integrons found in bacteria. It encodes for the integrase enzyme, which is responsible for the insertion and excision of gene cassettes within the integron structure (Partridge et al., 2001). Studies have shown that the presence of the *intl1* gene is strongly associated with the multidrug-resistant phenotype of clinical isolates of bacteria (Gillings et al., 2008). The *intl1* gene is one of the most prevalent types of integrons found in clinical isolates (Ding et al., 2019) and their presence has been associated with varying levels of pollution in the environment (Borruso et al., 2016). The widespread distribution of the *intl1* gene among different bacterial species has contributed to the rapid emergence and dissemination of antibiotic resistance, posing a serious threat to public health (Partridge et al., 2001).

For this research study, we were focused on characterizing the *intl1* gene sequences in three closely related *Pseudomonas* bacterial species: *Pseudomonas aeruginosa, Pseudomonas stutzeri and Pseudomonas putida.* All three of them are gram-negative bacterium widely distributed in the environment. *P. aeruginosa* is mostly isolated from clinical settings whereas the other two bacteria have been isolated from environmental samples. *P. aeruginosa* is known for its intrinsic resistance to many antibiotics. It is also a frequent opportunistic pathogen that can cause infections in humans, particularly in individuals with compromised immune systems (Gellatly & Hancock, 2013). Class 1 integrons are prevalent in clinical isolates of *P. aeruginosa* and have been implicated in the dissemination of antibiotic resistance genes in this species (Poirel et al., 2010). Studies have shown that class 1 integrons containing various antibiotic resistance gene cassettes are widespread among clinical isolates of *P. aeruginosa* (Sheikh et al., 2019).

Class 1 integrons are also frequently found in P. *stutzeri*, where they have been shown to play a role in the acquisition and dissemination of antibiotic resistance genes (Rocchetti et al.,

2019). Several studies have reported the presence of class 1 integrons containing various antibiotic resistance gene cassettes in P. *stutzeri* isolates from environmental and clinical sources (Collis & Hall, 1995; Gestal et al., 2011). Studies have reported the presence of class 1 integrons containing various antibiotic resistance gene cassettes in *P. putida* isolates from soil and water samples and the detection of the *intl1* gene, encoding the integrase enzyme responsible for class 1 integron insertion and excision (Agerso and Sandvag 2005; Huyan et al., 2020).

The purpose of this study is to characterize the *intl1* gene in these three *Pseudomonas* species and identify variation in each species. We are also interested in studying the evolutionary relationships between these bacteria based on this gene. Finally, as lateral gene transfer is one of the mechanisms the *intl1* gene sequences are transferred between bacteria we studied the location of this gene in the genome to observe patterns of inheritance of these genetic regions.
The high prevalence of class 1 integrons in these bacterial species highlights the importance of monitoring the dissemination of antibiotic resistance genes in this species and the potential role of these species as a reservoir for antibiotic resistance genes in the environment.

Methods

Accessing the data

The sequences used in this research project were obtained from the NCBI (National Center for Biotechnology Information) GenBank database (Agarwala et al., 2018). We restricted the search query to gene sequences for the *intl1* gene in the three *Pseudomonas* bacteria species. In total we found and downloaded 73 *intl1* gene sequences and their breakdown in each species were as follows: *P. aeruginosa* – 18 sequences (Supplementary Table 1), *P. putida* – 36 sequences (Supplementary Table 2a and 2b), and P. *stutzeri* – 17 sequences (Supplementary Table 3). Out of the 73 gene sequences certain gene descriptions indicated that they were

incomplete or plasmid sequences and those were excluded from the analysis. After removing those sequences, we had 43 complete gene sequences that were used for further data analysis.

Evolutionary Relationships

The Multiple Sequence Comparison by Log-Expectation tool (MUSCLE) (Edgar, 2004) was used to align all the *intl1* sequences. The data from MUSCLE was used to construct phylogenetic trees with the Molecular Evolution Genetic Analysis (MEGA X) (Kumar et al., 2018). The maximum-likelihood tree building model was used to generate the phylogenetic tree.

Synteny

Synteny is the property of genes in different organisms appearing in the same order in the genome (Sevillya et al., 2020). To generate the synteny we used the Mauve software (Darling et al., 2004). Flanking gene regions, 100 bps upstream and downstream of our gene of interest (*intl1*) were identified in NCBI database and those gene regions were downloaded in FASTA format. These FASTA sequences were inputted into the software to generate the synteny map

Identifying patterns

The gene regions from the synteny analysis were categorized into three major groups (Group 1,2 and 3) based on their position in the genome. These three categories were then superimposed on the phylogenetic tree that had been generated to identify patterns of inheritance in the data.

Results

The comparative genomics analysis for the *intl1* gene sequences indicated that the sequences are highly conserved among the three *Pseudomonas* species. The phylogenetic tree generated from the *intl1* sequences showed a predictable pattern of evolutionary relationships

(Figure 1). The clades separated out in a pattern where the *P. aeruginosa* sequences were most closely related to each other. Same was the case with the *P.stutzeri* and *P.putida* sequences.

Based on the synteny analysis we observed a pattern where the *intl1* gene was not found in the same genomic location for all the sequences **(Figure 2).** We categorized the sequences into three groups based on gene location: Group 1: *intl1* gene located in the middle, Group 2: *intl1* gene located towards the end and Group 3: *intl1* gene located in the beginning of the genomic region. This categorization was color-coded and super imposed on the phylogenetic tree generated in **Figure 1.** This phylogenetic tree with the color-coded categories is shown in **Figure 3.** We observe a pattern where the grouped categories do not follow the simple evolutionary relationship based on species relatedness. The position of *intl1* gene in the genome does not correlate with the species that it belongs to. Instead, we observed a random distribution that cannot be explained by evolutionary history.

Discussion

In this study, we aimed to categorize the *intl1* gene in three species of *Pseudomonas* bacteria and observe patterns of inheritance, as well as comparisons of the gene's genomic position. These gene sequences are highly similar as seen in the comparative genomics MUSCLE output. We were not surprised by this result as there is evidence to suggest that Class 1 integron sequences are highly conserved within Gammaproteobacteria class of bacteria (Zhang et al., 2018), which includes the *Pseudomonas* genus. The phylogenetic tree showed a predictable pattern distribution as the sequences belonging to the same bacteria species clustered together on the tree. Surprisingly, the distribution of the *intl1* gene within the genomic region did not follow the expected pattern: the gene position was not the same for the different bacterial

species. We predict that this could be due to class 1 integrons being embedded in transposons and plasmids, which allow their rapid dissemination through lateral gene transfer (Martinez-Freijo et al., 1998). Genes inserted through lateral gene transfer get inserted at random locations in the genome (Vogan and Higgs, 2011) and may have different gene positions within the same species based on mechanism of inheritance.

Class 1 integrons are commonly found in clinically important Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa* (Partridge, 2011). Several studies have demonstrated the prevalence of class 1 integrons in clinical isolates of bacteria. For example, a study conducted in Iran found a high prevalence of class 1 integrons in E. coli isolates from animal sources (Dehkordi et al., 2018). Similarly, a study in China found that multidrug-resistant *K. pneumoniae* isolates carried class 1 integrons (Wang et al., 2019).

The *intl1* gene is responsible for the formation of integrons, which are mobile DNA elements that can capture and incorporate gene cassettes containing antibiotic resistance genes (White et al., 2001). Integrons can transfer these resistance genes between different bacterial species, enabling the rapid spread of antibiotic resistance (Gillings et al., 2008). Studies have shown that the prevalence of *intl1* gene in clinical *Pseudomonas* isolates is strongly correlated with multidrug resistance (Zarei-Yazdeli et al., 2019).

Overall, the *intl1* gene is a major contributor to the global problem of antibiotic resistance, particularly in *Pseudomonas* bacteria. Understanding the mechanisms by which this gene promotes resistance is critical for developing new strategies to combat multidrug-resistant infections. With our research study we have shown that this gene is highly conserved in *Pseudomonas* and its position in the genome is highly variable. This pattern indicates the significant role of lateral gene transfer contributing to the spread of this gene in bacterial

populations. Future studies can expand this characterization of all *intl1* gene sequences within the *Pseudomonas* genus to generate a better picture of the spread and inheritance patterns of the gene. Also, the *intl1* gene can serve as a marker for studying the rate of lateral gene transfer in bacterial populations. Combined with the gene's role in the spread of antibiotic resistance it is imperative that we understand the mechanisms by which these integrons promote resistance. A better understanding of the prevalence of the *intl1* gene is critical for developing new strategies to combat multidrug-resistant infections.

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Figures

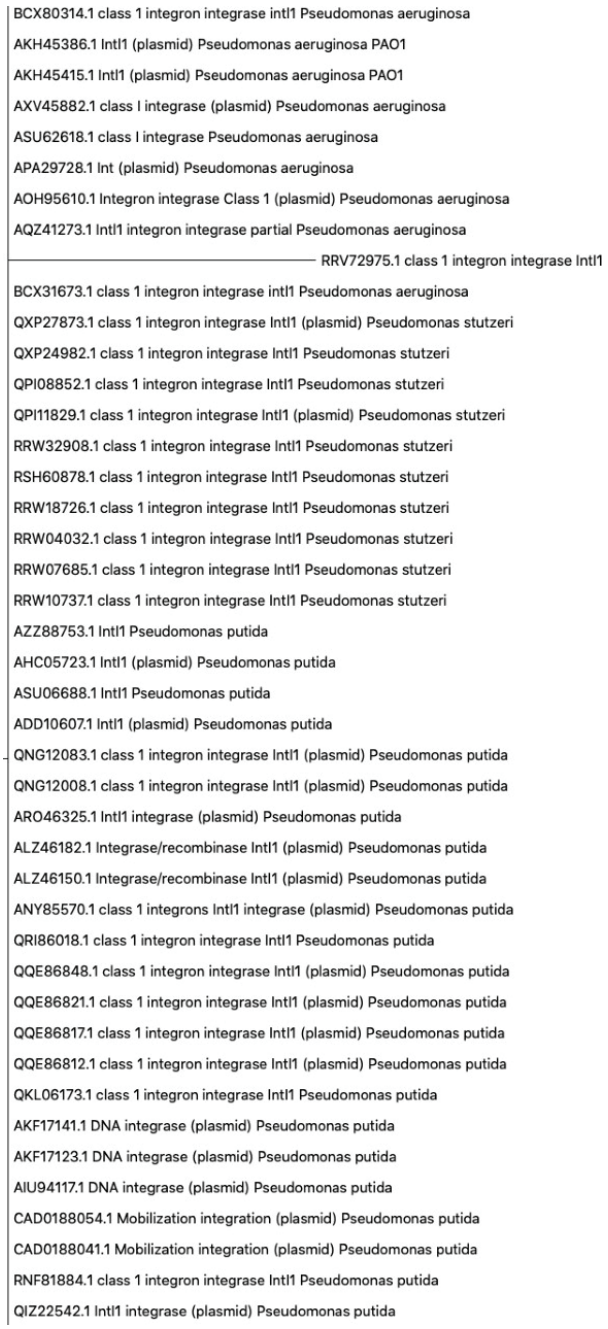

Figure 1. Phylogenetic tree showing the evolutionary relationships between three *Pseudomonas* species: *P. aeruginosa, P. stutzeri* and *P. putida*. The evolutionary history was inferred by using the Maximum Likelihood method. The tree with the highest log likelihood (-46782.5881) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

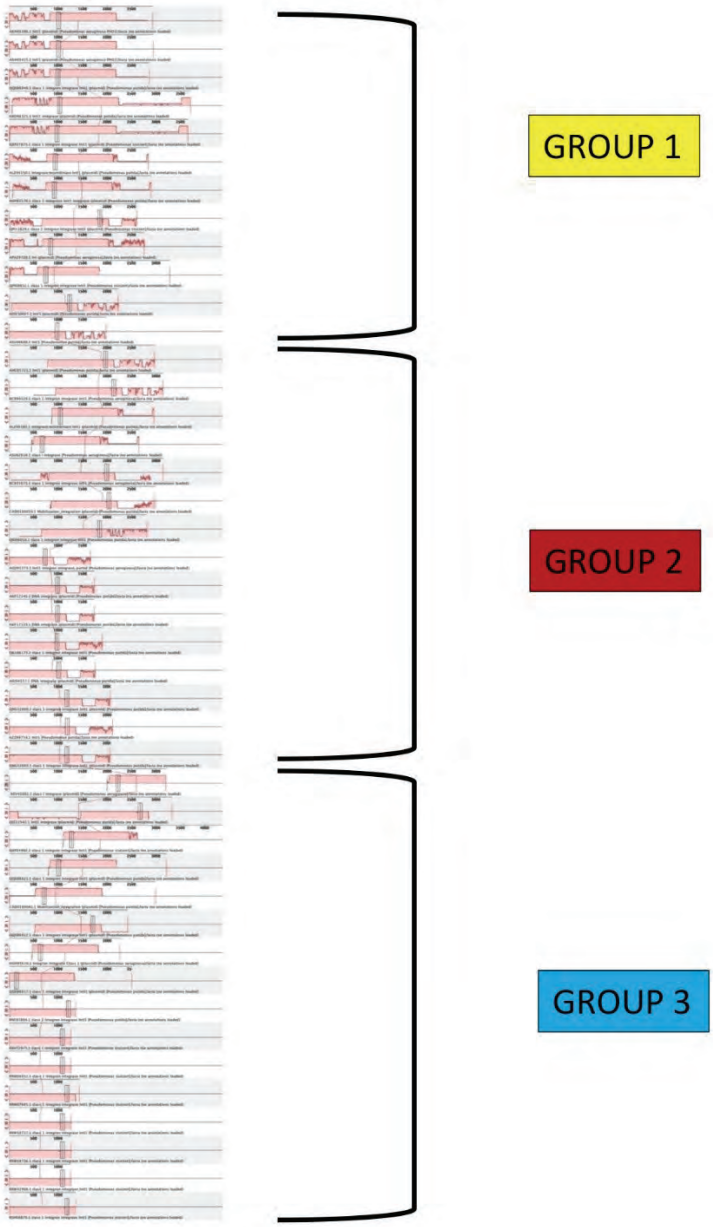

Figure 2. The synteny of 43 *intl1* gene sequences and the flanking genomic regions via Mauve software. The solid red line connecting each sequence shows the location of the matching section of the genome. The three groups are classifications based on position of the *intl1* gene in the genome. Similarity of genomic regions are based on the peak height and coloration patterns.

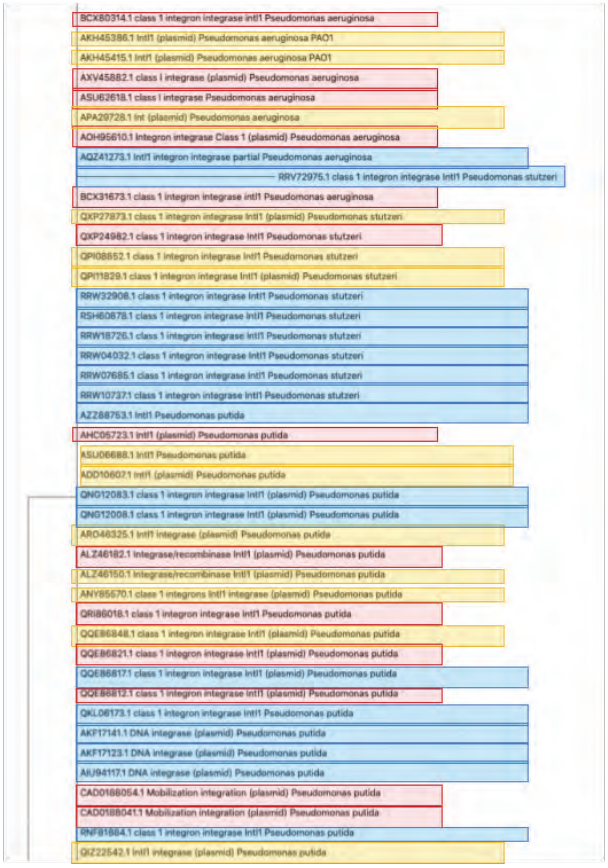

Figure 3. Phylogenetic tree showing the evolutionary relationships between three *Pseudomonas* species: *P. aeruginosa, P. stutzeri* and *P. putida*, superimposed by the groupings from the synteny analysis.

Supplementary Tables

#	Accession #	Description	Bacteria
1	AKH45415.1	Intl1	*Pseudomonas aeruginosa*
2	AKH45386.1	Intl1	*Pseudomonas aeruginosa*
3	AIB52522.1	Intl1	*Pseudomonas aeruginosa*
4	AIB52521.1	Intl1	*Pseudomonas aeruginosa*
5	BCX55635.1	integrase/recombinase	*Pseudomonas aeruginosa*
6	AXV45882.1	class 1 integrase	*Pseudomonas aeruginosa*
7	ASU62618.1	class 1 integrase	*Pseudomonas aeruginosa*
8	ARO39122.1	integrase	*Pseudomonas aeruginosa*
9	APA29728.1	Intl1	*Pseudomonas aeruginosa*
10	AOH95610.1	Integron intergase	*Pseudomonas aeruginosa*
11	AIA58908.1	integrase	*Pseudomonas aeruginosa*
12	BCX80321.1	class 1 integron	*Pseudomonas aeruginosa*
13	BCX80314.1	class 1 integron	*Pseudomonas aeruginosa*
14	BCX31673.1	class 1 integron	*Pseudomonas aeruginosa*
15	BCX80368.1	class 1 integron	*Pseudomonas aeruginosa*
16	BCX31665.1	class 1 integron	*Pseudomonas aeruginosa*
17	BCX31657.1	class 1 integron	*Pseudomonas aeruginosa*
18	AQZ41273.1	Intl1	*Pseudomonas aeruginosa*

Supplementary Table 1. Data table showing the class 1 integron (*intl1*) gene sequences used in the data analysis for *P. aeruginosa.*

#	Accession #	Description	Bacteria
1	ACT32121.1	IntI1	*Pseudomonas putida*
2	AZZ88753.1	IntI1	*Pseudomonas putida*
3	QZR95635.1	intI1	*Pseudomonas putida*
4	AHC05723.1	IntI1	*Pseudomonas putida*
5	ASU06688.1	IntI1	*Pseudomonas putida*
6	ADD10607.1	IntI1	*Pseudomonas putida*
7	ABQ65133.1	IntI1	*Pseudomonas putida*
8	QOD01666.1	class 1 integron	*Pseudomonas putida*
9	CZT31715.1	IntI1	*Pseudomonas putida*
10	QDK55174.1	IntI1	*Pseudomonas putida*
11	QNG12083.1	class 1 integron	*Pseudomonas putida*
12	QNG12008.1	class 1 integron	*Pseudomonas putida*
13	QNG09412.1	class 1 integron	*Pseudomonas putida*
14	ARO46325.1	IntI1 integrase	*Pseudomonas putida*
15	ALZ46182.1	Integrase/recombinase	*Pseudomonas putida*
16	ALZ46150.1	Integrase/recombinase	*Pseudomonas putida*
17	ANY85570.1	class 1 integron	*Pseudomonas putida*
18	QRI86018.1	class 1 integron	*Pseudomonas putida*

Supplementary Table 2a. Data table showing the class 1 integron (*intl1*) gene sequences used in the data analysis for *P. putida.*

#	Accession #	Description	Bacteria
19	QQE86848.1	class 1 integron	*Pseudomonas putida*
20	QQE86821.1	class 1 integron	*Pseudomonas putida*
21	QQE86817.1	class 1 integron	*Pseudomonas putida*
22	QQE86812.1	class 1 integron	*Pseudomonas putida*
23	QKL10228.1	class 1 integron	*Pseudomonas putida*
24	QKL06173.1	class 1 integron	*Pseudomonas putida*
25	AKF17141.1	DNA integrase	*Pseudomonas putida*
26	AKF17123.1	DNA integrase	*Pseudomonas putida*
27	AIU94117.1	DNA integrase	*Pseudomonas putida*
28	CZT31721.1	DNA integrase	*Pseudomonas putida*
29	CAD0188054.1	Mobilization_integration	*Pseudomonas putida*
30	CAD0188041.1	Mobilization_integration	*Pseudomonas putida*
31	CAK55555.1	integrase	*Pseudomonas putida*
32	ORL47611.1	class 1 integron	*Pseudomonas putida*
33	QIZ22805.1	IntI1	*Pseudomonas putida*
34	QIZ22790.1	IntI1	*Pseudomonas putida*
35	QIZ22558.1	IntI1	*Pseudomonas putida*
36	RNF81884.1	class 1 integron	*Pseudomonas putida*

Supplementary Table 2b. Data table showing the class 1 integron (*intl1*) gene sequences used in the data analysis for *P. putida* (continued from Supplementary Table 2a).

#	Accession #	Description	Bacteria
1	QXP27873.1	class 1 integron	*Pseudomonas stutzeri*
2	QXP24982.1	class 1 integron	*Pseudomonas stutzeri*
3	QPI08852.1	class 1 integron	*Pseudomonas stutzeri*
4	QPI11829.1	class 1 integron	*Pseudomonas stutzeri*
5	WP_019406427.1	class 1 integron	*Pseudomonas stutzeri*
6	RRW32908.1	class 1 integron	*Pseudomonas stutzeri*
7	RSH60878.1	class 1 integron	*Pseudomonas stutzeri*
8	RRW18726.1	class 1 integron	*Pseudomonas stutzeri*
9	RRW07685.1	class 1 integron	*Pseudomonas stutzeri*
10	RRW10737.1	class 1 integron	*Pseudomonas stutzeri*
11	RRW04032.1	class 1 integron	*Pseudomonas stutzeri*
12	RRW03744.1	class 1 integron	*Pseudomonas stutzeri*
13	RRV60008.1	class 1 integron	*Pseudomonas stutzeri*
14	RRV72975.1	class 1 integron	*Pseudomonas stutzeri*
15	RRV60001.1	class 1 integron	*Pseudomonas stutzeri*
16	OPG81442.1	class 1 integron	*Pseudomonas stutzeri*
17	RRV31657.1	class 1 integron	*Pseudomonas stutzeri*

Supplementary Table 3. Data table showing the class 1 integron (*intl1*) gene sequences used in the data analysis for *P. stutzeri.*

The Effect of Sex Education on Safe Sex Practices

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Abstract

This study looks to examine the relationship between sex education and the likelihood of college students engaging in safe sex practices. Sex education allows adolescents to gain information and skills needed to make the best decisions regarding sex and relationships (Planned Parenthood, n.d.). However, not all sex education is the same. Abstinence-only sex education teaches that refraining from sex until marriage is the expected sexual behavior of teens while comprehensive sex education provides medically accurate information about safe sex practices, including the use of contraceptives for reducing the chance of unwanted pregnancy (Abstinence Education, 2018). It is important for adolescents to receive sex education as it encourages youth to engage in safe practices such as sticking to sexual activity that does not spread infections, as well as understanding what a healthy sexual relationship can look like (Leung et al., 2019). Research shows that those who receive comprehensive sex education are more likely to practice safe sex when compared to those who received abstinence-only education. To examine this relationship, an online survey was distributed to undergraduate Cabrini University students. Results were presented at the Cabrini University Research Symposium.

Introduction

Sex education allows adolescents to gain information and skills needed to make the best decisions regarding sex and relationships (Planned Parenthood, n.d.). There are two forms of sex education used in the United States: abstinence-only sex education and comprehensive sex education. Abstinence-only sex education, sometimes referred to as "sexual risk avoidance," teaches that refraining from sex until marriage is the expected sexual behavior of teens. It excludes information about the use of contraceptives and condoms to prevent teenage pregnancy and sexually transmitted infections (STIs) (Abstinence Education, 2018). In contrast, comprehensive sex education, sometimes referred to as "evidence-based sex education," provides medically accurate information about safe sex practices, including contraceptives and condoms as effective ways to reduce unintended pregnancy and STIs. This also includes details on forming healthy relationships, communication skills, and human development (Abstinence Education, 2018). Sex education plays a major role in adolescents' sexual behaviors and risk

avoidance as it can give adolescents the skills needed to increase safe sex practices such as using consistent contraceptive use and having knowledge on STIs (Planned Parenthood, n.d.).

A study found that abstinence-only sex education is positively correlated with teenage pregnancy and higher rates of STIs despite its focus on refraining from sexual activity (Probst, 2021). This study determined that those who received comprehensive sex education were more likely to use contraceptives and had a delay in sexual debut (Probst, 2021). Comparably, another study found those who received comprehensive sex education were less likely to report having vaginal intercourse and were 50% less likely to report a teenage pregnancy (Kohler et al., 2008). In addition, those who received comprehensive sex education were significantly more likely to use safe sex communications such as discussing the desire to use contraceptives (Grasso & Trumbull, 2021). They were also more likely to have positive attitudes and intentions about practicing safe sex when compared to those who received abstinence-only sex education (Walcott et al., 2011).

It is important for adolescents to receive medically accurate sex education as it encourages safe sex practices such as using condoms, getting tested for STIs, and sticking to sexual activity that does not spread infections such as masturbation (Planned Parenthood, n.d.). It also ensures that children learn how to avoid coercion and abuse by educating them on their anatomy (Dyasi, 2021). Furthermore, medically accurate sex education allows youth to gain knowledge of the reproductive system, and the prevention of STIs (Leung et al., 2019). It encourages youth to better understand sexual relationships and increases their physical, mental, emotional, and social well-being regarding sexuality (Leung et al., 2019). Lastly, it promotes an understanding of what healthy sexual relationships can look like for youths (Leung et al., 2019).

Literature Review

Sex education plays a key role in adolescents' sexual behaviors and sexual risk avoidance (Planned Parenthood, n.d.). With the United States federal government primarily funding abstinence-only programs, American teens are not receiving medically accurate information needed to engage in safe sex practices (Federally Funded, 2021). With abstinence-only sex education programs primarily focusing on refraining from sex until marriage, it excludes information on contraceptives (Abstinence Education, 2018). This can lead to an increase in teen pregnancy and STI diagnoses in adolescents and young adults who receive this type of sex education. On the other hand, comprehensive sex education allows teens to acquire information on contraceptive use, STI knowledge, and details on forming healthy relationships and sexual communication skills (Abstinence Education, 2018). This gives adolescents the skills needed to increase safe sex practices such as delaying the age of sexual debut, increasing sexual communication, increasing STI knowledge, increasing contraceptive use, increasing the frequency of STI testing, and not having a teenage pregnancy or STI diagnosis (Coles et al., 2011; Contraception, 2022; Rotz et al., 2020; Subbarao, 2017).

Age of Sexual Debut

Delaying the age of sexual debut is an important aspect of safe sex because it is positively correlated with reducing the chances of teenage pregnancy or contracting an STI as an adolescent (Rotz et al., 2020). Delaying the age of sexual debut is linked to improving stability in youths' romantic relationships by allowing them time to consider their expectations of a romantic partner (Rotz et al., 2020). Research shows that with comprehensive education providing lessons that include negotiation skills, how to handle peer pressure, and how to say no to sex, it is correlated with a delay in the age of sexual debut (Probst, 2021). Furthermore, research found that

comprehensive programs that emphasize both abstinence and contraceptive use have a significant impact on delaying the initiation of sexual activity (Kirby & Laris, 2009). Additionally, an evaluation conducted on the impacts of abstinence programs on teen sexual activity found no significant impact on delaying the age of sexual debut in teens who received abstinence-only education (Trenholm et al., 2008). In opposition to this evidence, separate research found those who received abstinence-only sex education were significantly older when they engaged in sex for the first time (Grasso & Trumbull, 2021). Moreover, a different study found no statistical difference in delaying sexual activity in those who received comprehensive sex education and those who received abstinence-only sex education (Pittman & Gahungu, 2006). Despite the lack of consensus in these studies, it is crucial for adolescents to receive the knowledge needed to make informed decisions on when to engage in sex for the first time because delaying this age can have a significant impact on a teen's future (Rotz et al., 2020).

Sexual Knowledge and Communication

While sexually transmitted infections are reported by people of all ages, ages 16 to 24 have been considered to be at a significantly higher risk for STIs (Subbarao, 2017). This age group accounts for nearly half of the 26 million new sexually transmitted infections that occurred in the United States in 2018 (CDC, 2021). This is likely due to young people having higher rates of unprotected sex and more sexual partners than other age groups (Subbarao, 2017). As such, it is important for adolescents to receive sex education that teaches about the dangers of STIs and how to best protect oneself from becoming infected. Those who received comprehensive sex education were more likely to be aware of the dangers of sexually transmitted diseases and felt more sexually responsible (Pittman & Gahungo, 2006). In addition, those who receive comprehensive sex education have significantly higher human immunodeficiency virus (HIV)

knowledge when compared to those who received abstinence-only education due to it including medically accurate lessons on STIs (Walcott et al., 2011).

Another aspect of sexual knowledge is possessing the skills needed for healthy sexual communication. Developing communication skills while in sex education can have a positive impact on adolescents' ability to have safer sexual decision making such as delaying initiation of sex and the desire to use contraceptives with their partner (Grasso & Trumbull, 2021). Lessons can include information on consent and communication about previous STI diagnoses (Grasso & Trumbull, 2021). Many feel as though having these conversations can "ruin the mood." However, teaching adolescents that sexual communication can build trust with their partners, and lowers the risk of pregnancy, can encourage youth to have these important conversations. Those who received comprehensive sex education were significantly more likely to use safe sex communications (Grasso & Trumbull, 2021). Additionally, those who received lessons on negotiation skills and how to handle peer pressure predicted more positive attitudes and intentions about practicing safe sex with partners (Walcott et al., 2011).

Contraceptive Use

Using contraceptives such as condoms and intrauterine devices is an important aspect of practicing safe sex as it can lower the risk of unwanted pregnancy and can help prevent sexually transmitted infections (Contraception, 2022). Young people report higher rates of unprotected sex; however, this behavior can change based on the type of sex education they received. For example, comprehensive education increased the likelihood of contraceptive use and caused a reduction in risky sexual behaviors (Probst, 2021). Likewise, women who received comprehensive sex education were more likely to use an effective method of contraception during their first sexual engagement than those who did not receive this type of education

(Cheedalla et al., 2020). Males are more likely to report dual contraceptive use if they received comprehensive sex education (Jaramillo et al., 2017). Additionally, sex education that included negotiation skills predicted more consistent condom use (Walcott et al., 2011). Lastly, programs that emphasized both abstinence and contraceptive use significantly increased contraceptive use in adolescents (Kirby & Laris, 2019).

Teen Pregnancy and STI Diagnoses

Non-consistent use of contraceptives can lead to an increase in teenage pregnancy (Coles et al., 2011). Without lessons on how to properly avoid unwanted pregnancy such as refraining from sex, and the importance of contraceptive use, adolescents have a higher chance of becoming pregnant and or getting a partner pregnant (Coles et al., 2011). In terms of pregnancy, adolescents who received abstinence-only sex education are positively correlated with becoming pregnant (Probst, 2021). Additionally, reports of teenage pregnancy are 50% lower in those who received comprehensive sex education than in those who did not (Kohler et al., 2008). This is likely due to comprehensive sex education providing medically accurate information regarding abstinence and other safer sex practices including condom use as effective ways to reduce unintended pregnancy (Abstinence Education, 2018).

Another consequence of inconsistent contraceptive use is contracting an STI such as Chlamydia, HIV, and Gonorrhea. Those who receive comprehensive sex education are less likely to be diagnosed with an STI and are more likely to get tested for STIs (George et al., 2022). For instance, a study on exposure to comprehensive sex education found a reduction in being HIV-positive in adolescent girls. This study also found that these adolescent girls were more likely to get tested for HIV if they received comprehensive sex education (George et al., 2022). Additionally, those who received comprehensive sex education were significantly more likely to

report prior STI testing (Craig-Kuhn et al., 2020). Comparably, a study found that those who received abstinence-only sex education were positively correlated with higher rates of STIs (Probst, 2021). However, a few studies have disputed the idea that comprehensive sex education reduces the rate of STIs. For example, one study found no relationship between abstinence programs and STI rates (Trenholm et al., 2008).

Hypothesis

Those who received comprehensive sex education are more likely to engage in safe sex practices when compared to those who received abstinence-only sex education.

Methodology

Sample and Procedure

A nonprobability, availability sampling method was used to collect data for this study. Data was collected from N= 60 participants from Cabrini University. This study intended to find a relationship between sex education and the likelihood of college students engaging in safe sex practices. Electronic surveys were distributed to undergraduate Cabrini University students and athletes through professors to be completed on their personal laptops or cellphones in the spring semester of 2023. Before distributing the electronic surveys, the researcher received approval from Cabrini University's Institutional Review Board (IRB).

Demographics

This sample consisted of 76.3% (n=45) cisgendered female respondents, 18.6% (n=11) cisgendered male respondents, 1.7% (n=1) nonbinary/third gender respondents, 1.7% (n=1) genderqueer respondents, and 1.7% (n=1) of respondents prefer not to say. The racial distribution

of the sample was composed of 69.5% (n=41) White, 13.6% (n=8) Asian, 10.2% (n=6) Black or African American, 5.1% (n=3) American Indian or Alaskan Native, and 1.7% (n=1) Native Hawaiian or other Pacific Islander. The ethnicity of respondents consisted of 78% (n=46) Non-Hispanic/Latino/a and 20.3% (n=12) Hispanic/Latino/a. The age of respondents composed of 33.9% (n=20) 21-year-olds, 23.7% (n=14) 20-year-olds, 18.6% (n=11) 19-year-olds, 11.9% (n=7) 22-year-olds, 10.2% (n=6) 18-year-olds, and 1.7% (n=1) 23-year-olds. Lastly, the class year consisted of 37.3% (n=22) juniors, 23.7% (n=14) seniors, 18.6% (n=11) sophomores, and 18.6% (n=11) freshman. See table 1 for complete breakdown.

Table 1
Sample Demographics

	Frequency	*Valid Percent*
Gender		
Cisgender Female	45	76.3
Cisgender Male	11	18.6
Non-Binary/Third-Gender	1	1.7
Gender Queer	1	1.7
Prefer Not to Say	1	1.7
Race		
White	41	69.5
Black or African American	6	10.2
American Indian/Alaskan Native	3	5.1
Asian	8	13.6
Native Hawaiian or Other Pacific Islander	1	1.7
Age	6	10.2
18 years old	11	18.6
19 years old	14	23.7
20 years old	20	33.9
21 years old	7	11.9
22 years old	1	1.7
23 years old		
Class Year	11	18.6
Freshman	11	18.6
Sophomore	22	37.3
Junior	14	23.7
Senior		
Ethnicity	12	20.7
Hispanic or Latino/a	46	79.3
Non-Hispanic or Latino/a		

Variables of Interest

The independent variables for this study are receiving sex education in high school and the type of sex education received. Sex education is defined as "teaching and learning about a broad variety of topics related to sex and sexuality" (Planned Parenthood, n.d.). Participants were asked 3 questions. Participants were asked, "Did you receive sex education in high school?" (1. Yes, 2. No) 74.6% (n=44) answered yes. Next, participants were asked "Did you receive abstinence-only sex education in high school? This teaches that refraining from sex until marriage is the expected sexual behavior of teens." (1. Yes, 2. No) 71.2% (n=42) of respondents answered no. Lastly, participants were asked "Did you receive comprehensive sex education in high school? This provides medically accurate information about abstinence and other safe sex practices, including contraceptives and condoms as effective ways to reduce unintended pregnancy and sexually transmitted infections." (1. Yes, 2. No) 62.7% (n=37) answered yes. See table 2 for the complete breakdown.

Table 2

Independent Variables

	Frequency	***Valid Percent***
Sex Education		
Yes	44	77.2
No	13	22.8
Abstinence		
Yes	13	23.6
No	42	76.4
Comprehensive		
Yes	37	63.8
No	21	36.2

The dependent variable of this study is engaging in safe sex. This study defines engaging in safe sex as delaying the age of sexual debut, using sexual communication, using

contraceptives, having STI knowledge, and lastly getting tested for STIs. Participants were asked 7 questions to assess safe sex practices. The first 2 questions were asked to assess the age of sexual debut. The first question asked was "Have you ever had sexual intercourse? Defined as penetration of the vagina/anus by a penis (National Youth Risk Behavior Survey, 2017)." (1. Yes, 2. No) 74.6% (n=44) of respondents answered yes. The second question was "How old were you when you had sexual intercourse for the first time? (National Youth Risk Behavior Survey, 2017)" (1. 14 years or younger, 2. 15 years old, 3. 16 years old, 4. 17 years older, 5. 18 years or older) 35.6% (n=21) answered 18 years or older.

Next, to assess contraceptive use participants were asked 1 question. Participants were asked, "The last time you had sexual intercourse, did you or your partner use contraceptives? Ex: condoms, IUD or implant, birth control pills, etc. (National Youth Risk Behavior Survey, 2017)." (1. Yes, 2. No and I do not know) 64.4% (n=38) answered yes. To assess sexual communication use participants were asked 1 question. Participants were asked, "I initiate the topic of safer sex with my potential sexual partner. (Dilorio et al., 1992)" (1. Never, 2. Sometimes, 3. Most of the time) 44.1% (n=26) of respondents answered most of the time. For STI knowledge participants were asked 1 question. This question asked "What are the body fluids that increase the risk for sexually transmitted infections through (STIs) transmission? Choose all that apply. (Drago et al., 2016)." (1. Blood, 2. Urine, 3. Saliva, 4. Sweat, 5. Semen) 78% (n=46) answered incorrectly. Lastly, for the frequency of STI testing participants were asked 2 questions. The first question asked "Have you ever been tested for sexually transmitted infections (STIs)? (CDC, 2021)." (1. Yes, 2. No) 50.8% (n=30) of respondents answered no. The last question asked, "How often do you get tested for sexually transmitted infections (STIs)?" (1.

I have never been tested for STIs, 2. 1-2 times a year, 3. 3-4 times a year) 47.5% (n=28) of respondents answered I have never been tested for STIs. See table 3 for complete breakdown.

Table 3

Dependent Variables

	Frequency	***Valid Percent***
Sexual Intercourse		
Yes	44	74.6
No	15	25.4
Age of Debut		
14 years or younger	3	6.7
15 years old	10	22.2
16 years old	9	20.0
17 years old	2	4.4
18 years or older	21	46.7
Contraceptives		
Yes	38	77.6
No and I don't know	11	22.4
Communication		
Never	11	22.0
Sometimes	13	26.0
Most of the Time	26	52.0
STI Knowledge		
Correct	11	19.3
Incorrect	46	80.7
Tested for STIs		
Yes	27	47.4
No	30	52.6
How often Tested		
I Have Never Been Tested for STIs	28	63.6
1-2 Times a Year	14	31.8
3-4 Times a Year	2	4.6

The table below is a descriptive statistics table. It provides an overview of all variables of interest. See Table 4 for complete breakdown of Descriptive Statistics. It is important to note that response rates varied per variable due to time limitations or content sensitivity.

Table 4

Variable Descriptive Statistics

Variable	*N*	*Mean*	*Median*	*Mode*	*Range*	*Standard Deviation*
Gender	59	-	-	1	4	-
Age	59	-	-	21	5	-
Class Year	58	-	-	3	3	-
Race	59	-	-	1	4	-
Ethnicity	58	-	-	2	1	-
Sex Education	57	-	1.0000	1.00	1.00	-
Abstinence	55	-	2.0000	2.00	1.00	-
Comprehensive	58	-	1.0000	1.00	1.00	-
Sexual Intercourse	59	-	1.00	1	1	-
Age of debut	45	-	4.00	5	4	-
Contraceptives	49	-	1.0000	1.00	1.00	-
Communication	50	-	3.00	3	2	-
STI Knowledge	57	-	2.00	2	1	-
Tested for STIs	57	-	2.00	2	1	-
How Often Tested	44	-	1.00	1	3	-

Plans for analysis

To analyze the relationship between sex education and college student's safe sex practices, a Spearman Correlation was run to test the hypotheses: Those who received comprehensive sex education are more likely to engage in safe sex practices when compared to those who received abstinence-only sex education. A Spearman correlation was run due to the variables being binary and ordinal.

Results

Table 5 below shows the results of the Spearman correlation that was conducted to examine the relationship between sex education and college students' safe sex practices. In the Spearman correlation, there is one significant relationship. As seen in table 5, there is a weak positive relationship between receiving sex education and using contraceptives the last time you had sexual intercourse: $r_s(58) = .292$, $p < .05$. There is a variance of 84.64%. This concludes that receiving sex education in high school, regardless of the type, is positively associated with college students using contraceptives the last time they had sexual intercourse. The results of this study did not support the hypothesis that stated that those who received comprehensive sex education are more likely to engage in safe sex practices when compared to those who received abstinence-only sex education. Unfortunately, no other significant relationships were found between the three independent variables of sex education and safe sex practices

Table 5

Spearman Correlation between Sex Education and Safe Sex Practices

Variables	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*	*(6)*	*(7)*	*(8)*	*(9)*	*(10)*
1) Sex Education	1.000	-	-	-	-	-	-	-	-	-
2) Abstinence	-3.15*	1.000	-	-	-	-	-	-	-	-
3) Comprehensive	.729**	-.378**	1.000	-	-	-	-	-	-	-
4) Age of Debut	-.016	.086	-.048	1.000	-	-	-	-	-	-
5) Sexual Intercourse	.150	.030	-.006	.149	1.000	-	-	-	-	-
6) Communication	.108	-.201	-.049	.176	.098	1.000	-	-	-	-
7) STI Knowledge	.136	-.166	.070	-.029	.160	-.040	1.000	-	-	-
8) Tested for STIs	-.091	.109	-.139	.465**	.541**	.176	-.027	1.000	-	-
9) How often Tested	.121	.082	.169	-.624**	-.511**	-.242	-.031	-.866**	1.000	-
10) Contraceptives	.292*	-.282	.214	-.078	.627**	-.109	.137	.019	-.185	1.000

Discussion

The purpose of this study was to examine the relationship between the type of sex education received and the likelihood of college students engaging in safe sex practices. Based on the findings of previous literature, one hypothesis was formed: Those who received comprehensive sex education are more likely to engage in safe sex practices when compared to those who received abstinence-only sex education. The results found in the Spearman correlation did not support this hypothesis but noted that receiving sex education in high school was positively correlated with college students using contraceptives with their last sexual partner. This concludes that the two types of sex education studied, comprehensive sex education and abstinence-only sex education, were not positive predictors of engaging in safe sex practices, but receiving sex education, regardless of the type, was a positive predictor of contraceptive use. This is likely due to sex education as a whole playing a major role in adolescents' sexual behaviors and risk avoidance, as it allows adolescents to gain information and skills needed to make the best decisions regarding sex (Planned Parenthood, n.d.). Although research suggests that those who receive comprehensive sex education are more likely to engage in safe sex practices such as contraceptive use, one study suggests that programs that emphasized both abstinence and information regarding contraceptives significantly increased contraceptive use in adolescents (Kirby & Laris, 2019). Meaning that sex education, regardless of its focus, can be a positive predictor in adolescents and young adults engaging in safe sex. No other significant relationship was found between the type of sex education received and engaging in other forms of safe sex practices such as delaying the age of sexual debut, using sexual communication, having STI knowledge and increasing the frequency of STI testing.

Some major limitations of this study include sample size affected by timing restrictions

and content sensitivity. This study used a nonprobability, availability sampling method to collect data from N= 60 participants from Cabrini University. It is important to note that two survey questions were excluded: “When you were under the age of 21 did you or one of your sexual partners ever become pregnant?” and “Have you ever been diagnosed with a sexually transmitted infection (STIs) in the past?” These were excluded from the final analysis due to no variation in responses, and thus outliers were removed from the study.

Additionally, it is necessary to state that the sample in this study is not representative of the overall population of Cabrini University students. Cisgendered females were overrepresented in this sample with a percentage of 76.3% and cisgendered males were underrepresented with a percentage of 16.6% when compared to the overall gender demographics of Cabrini University that reports a percentage of 63.6% cisgendered females and 36.4% cisgendered males (Cabrini University, n.d.). In addition, White students were overrepresented in this study with a percentage of 69.5% as well as Hispanic students with a percentage of 20.3%. However, Black students were underrepresented with a percentage of 10.2%. When compared to the racial and ethnicity demographics collected by College Factual, White students make up 57.7% of Cabrini University's overall population, while Black students represent 19.4% and Hispanic students represent 10.5% of the population (Cabrini University, n.d.). Lastly, 21-year-old students were overrepresented in this study at 33.9% when compared to the 24.9% reported (Cabrini University, n.d.). However, the majority of students in Cabrini University's research methods class are primarily made up of 20-21 year old students who are required to respond to the surveys distributed by their peers. Due to the small sample size, it was likely that this sample would not be representative of the overall population at Cabrini University. Results of this study were likely skewed by this small sample size, as small sizes can undermine the validity of the

study (Faber & Fonseca, 2014). Another limitation of this study included rushed timing, as the collection of data had to be completed by a certain date that excluded potential responses that may have been collected with a longer timeframe. Research suggests that response levels often do increase with longer timeframes (Walentynowicz, 2018).

Moreover, it is important to note that only 38 percent of high schools in the United States teach all 19 topics identified as critical for sex education by the Centers for Disease Control and Prevention (Janfaza, 2020). These topics included "providing adolescents the essential knowledge and critical skills needed to avoid HIV, other STDs, and unintended pregnancy" which are all key factors of comprehensive sex education (Brener et al., 2017, p. 3). However, in this sample 62.7% of respondents reported receiving comprehensive sex education, meaning those who received comprehensive sex education in high school are overrepresented in this sample when compared to the overall population.

Additionally, content sensitivity played a major role in the final results of this study. With Cabrini University being a Catholic institution, participants may have limited their responses to questions regarding sexual intercourse and other sex practices as it may be "out of bounds for ordinary conversation" (Yan, 2021). Furthermore, these types of sensitive questions may have offended or discouraged respondents due to the fear of disclosure of responses by the researcher, despite the consent form of the survey discussing the confidentiality of results (Yan, 2021). Since questions asked by the researcher included possible "deviant" sexual behaviors such as engaging in sex before the age of 18 or having an STI diagnosis, participants may have been discouraged to respond as this goes against societal norms or religious attitudes (Yan, 2021).

In conclusion, implications of this study can include future research on how sex education affects college student's contraceptive use. To best study how sex education affects

college student's sexual behaviors in the future, researchers should have access to a longer collection period for increased responses and have access to a more diverse sample that accurately represents the target population. Additionally, to expand literature on this topic, future research should examine the effects of sex education on college students in both religious and non-religious colleges as this difference has not been previously described. Lastly, this study can be used to justify that sex education classes should be offered at the college level to further expand college students' sexual knowledge and increase their likelihood of engaging in other safe sex practices besides contraceptive use.

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Media Consumption and the Belief in Intimate Partner Violence Myths

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Abstract

Myths regarding intimate partner violence (IPV) govern the societal perspective of victims and offenders. IPV myths dictate how society will view this, which has a detrimental impact on victims and fundamental resources needed to re-enter society. Myths and misconceptions regarding intimate partner violence are evident within platforms of the media, such as television shows, social media, and news stories. With the growing popularity and usage of the media, society adopts these myths portrayed within the media as societal knowledge (Tsfati & Cohen, 2013). Based on one's level of media consumption, regardless of the type of platform, they adopt these myths as their own beliefs and perceptions continuing the impact on victims and their voices. Additionally, violent themes that are consumed within media consumption has been evaluated to increase an individual's acceptance of violent-based beliefs (Gavin & Kruis, 2022). The purpose of this study is to evaluate the relationship between a student's level of media consumption and media violence, with their level of agreement with a popular intimate partner violence myth. This study utilized quantitative research through the use of electronic surveys that were collected from 79 undergraduate students at Cabrini University in the Spring of 2023. It was hypothesized that the higher level of media consumption, the more a student will be likely to agree with the IPV myth stated. Results show that there is not a significant relationship between these 3 variables.

Introduction

Intimate partner violence (IPV) is an ongoing public health issue within contemporary society. Intimate partner violence characterizes the type of abuse that occurs between previous and current spouses or dating partners (Centers for Disease Control and Prevention [CDC], 2022). IPV encompasses a range of abusive behaviors but is not limited to physical, sexual, or psychologically harmful acts. Statistically, around 41% of women and 26% of men have experienced a form of violence perpetuated by a romantic partner in their lifetime, otherwise calculated as 1 in 3 women and 1 in 4 men (CDC, 2022). Adversely, 61 million women and 53 million men reported an experience of psychological abuse by an intimate partner in their lifetime (CDC, 2022), statistics that demonstrate the demand for recognition. IPV often occurs before the age of 25 years old therefore evaluating the perceptions of IPV in college students and variables that may influence their beliefs and perceptions is valuable to existing and future research (Wilson & Smirles, 2022).

There has been a growing recognition of intimate partner violence within research due to a paradigm shift in societal beliefs pertaining to the characteristics of this violence, such as the gender of the victim or offender (Stephenson et al., 2022). Within this heightened awareness, misconceptions related to IPV have come to fruition as myths in society. IPV myths have a strong influence on societal knowledge and are sustained through gender norms and social stereotypes (Westbrook, 2009). These myths are encouraged through societal constructs such as the media which plays a powerful role in the encouragement of these myths based on their inaccurate portrayal. Various platforms of media, such as social media, television, and the news, provide its function to cater to specific individualistic needs, such as entertainment or information, that allows it to be widely accessible. These different platforms, and media consumption, establish its ability to reach large populations that expose society to societal myths. Through the producing of IPV within the media, the narrative is structured around societal myths that separates it from reality (Tualeka & Bungin, 2020). They also capture violent and negative attitudes that are evident within intimate partner violence. The media acts as a catalyst in the transmission of societal information and myths; therefore, it is important to evaluate an individual's engagement with the media, to understand their susceptibility to internalizing these perceptions.

Based on one's consumption of the media with the consistent exposure to myth-based knowledge, or certain beliefs, individuals are subjected to adapting these perceptions as their own (Tsfati & Cohen, 2013). As a counterpart to media consumption, violence within media platforms or the construct of violence is an additional concern related to IPV myths. Violent behaviors or attitudes within the media have been evaluated to be an encouraging element of intimate partner violence myths as they normalize violent-based beliefs and attitudes that are a

construct of violence (Huesmann, 2007). Based on the power of sexist, gendered based violence that is conceptualized within media platforms, George Gerbner's 1998 Cultivation Theory developed the idea of the "mean world syndrome." Within this theory, he evaluates how the mass production of violent media that encompasses various negative views, instigates a reflection in society of similar thoughts and behaviors (Gerbner, 1998). Gerbner's theory provides an explanation for the distortion in one's belief influenced by societal myths that are based on their consumption of violent media. Additionally, Social Cognitive Theory provides a deeper understanding of the media's powerful influence on individual perceptions based on the theory that thoughts and behaviors are developed through the modeling of others (Toplu-Demirtas et al., 2020).

The myths that exist within a society that are mass-produced through platforms of the media are important to examine due to the impact it has on the awareness and acknowledgment of intimate partner violence. There is little research that has been done to evaluate the relationship between media consumption and the belief of intimate partner violence myths therefore it is important to conduct this study to expand on the current body of literature. It is valuable to determine the media's position in the continuation of IPV myths due to its growing popularity in contemporary society. This study seeks to evaluate the impact an individual's level of media consumption has on their beliefs in intimate partner myths that exist within society.

Literature Review

Media Consumption

The evolution of the media from traditional platforms such as newspapers to modern-day technology has led to a widespread increase in media consumption based on its availability and

accessibility (Tana et al., 2020). The media's influence on societal and individual beliefs is driven by the level of consumption and engagement with the media (Tsfati & Cohen, 2013). The media is positioned in society as the means for the transfusion of societal ideologies, attitudes, and perceptions; therefore, in this role, societal knowledge as a reflection of societal myths becomes an individual's personal perceptions (Carlyle et al., 2014). These notions are consistent across each platform of the media that consumers are exposed to and are a part of the collective social stratification. The level of consumption of media platforms attributes to an individual's likelihood of developing similar thoughts and opinions (Tsfati & Cohen, 2013). Individuals assimilate their beliefs with societies and apply them to their own social constructs (Gavin & Kruis, 2022). Societal beliefs can reflect myths that, although are a distortion of reality, are reinforced through the media (Carlyle et al., 2014). The media has the ability to conform IPV through a stereotypical lens to downplay the reality behind the violence and support existing popular myths. An individual's perspective is then derived from the media's narrative of IPV that exists as the continuation of myths.

Violent Media

Previous research has denied a relationship between violent media and its effect on committing violent behavior; however, it has discovered a correlation between violent media consumption and becoming more accepting of violent beliefs and perceptions (Gavin & Kruis, 2022). Media violence as it pertains to IPV centers it focus on the depiction of women within the media that encourages sexist views. The portrayal of women within the media is primarily as victims and sex objects through platforms such as pornography, sexual-based movies and music videos, and content on social media (Gavin & Kruis, 2022). This constructed view of women has been understood to influence societal attitudes that are rooted in violence and to be accepting of

IPV behaviors (Gavin & Kruis, 2022). The literature demonstrates how violent themes within the media contribute to the sexual outlook on women and the acceptance of violence that is predisposed within IPV.

Consumption of violent media leads to a reflection in individuals' perceptions and beliefs regarding intimate partner violence as it normalizes violent-based attitudes. It desensitizes society to the perpetuation of violence as if it's normality of societal culture (Kohlman et al., 2014). The deviated acceptance of intimate partner violence behaviors stems from exposure to violent and aggressive media programs that justifies certain negative behaviors (Gavin & Kruis, 2022). Higher levels of violent media consumption introduce a normalization of violent behaviors or beliefs related to intimate partner violence and promotes individuals to internalize these behaviors within their own relationships.

Intimate Partner Violence Myths

Intimate Partner Violence myths are deeply rooted in historical feminist-based ideologies that are characterized by male power, control, and dominance (Jankowski et al., 2018). Gender-stereotypes and social constructs have prolonged gender inequalities that are illustrated within myths of intimate partner violence. The myths surrounding this topic contribute to the normalization of intimate partner violence in society by excluding the culpability of the offender, patronizing the victim, and desensitizing individuals to the public health concerns intimate partner violence continues to be (Fleming & Franklin, 2020). Additionally, these myths encourage a continuum of the silence of victims of intimate partner violence of any sexual-oriented relationship, gender, race, etc., to maintain a society driven by systemic inequalities they are derived from.

Societal myths of intimate partner violence are shared knowledge of individuals in society that, although are being recognized, previous literature has introduced a separate branch of intimate partner violence myths titled, "information myths," (Westbrook, 2009, p. 826). These "information myths" are internalized beliefs held by victims, survivors, public services members, etc., that are not labeled as myths but are a personal taxonomy. Although this study discusses societal myths regarding intimate partner violence, it is important to highlight the significant literature on this topic as it concerns the experience of intimate partner violence victims. The creation of "information myths" that exists separate from societal myths demonstrates how damaging intimate partner violence is to victims as they endure secondary victimization in believing in a false narrative. In a previous study (e.g., Westbrook, 2009), women discussed having their own "information myths" such as, the abuse is only considered abuse if there's a physical injury or mark, IPV violence only occurs among uneducated individuals, and victims or offenders of intimate partner violence are mentally ill. "Information myths" establish a personal narrative that exists as a re-victimization.

The term intimate partner violence includes various abusive behaviors that can either be physical or nonphysical such as psychological vs. sexual (CDC, 2022). Abusive physical behaviors are easier to understand in comparison to nonphysical or psychological abuse as individuals in society lack the awareness of the diversity of this type of violence (Minto et al., 2022). Recognizing intimate partner violence is difficult in the interpretation of nonphysical abuse as these behaviors are easily confused with a "controlling, manipulative" personality of the offender that excludes their guilt of abusing their partner; however, it is just as damaging and harmful as physical violence (Minto et al, 2022, p. 2419). The lack of knowledge behind nonphysical intimate partner violence behaviors establishes this myth that abuse may only be

physical and these psychologically abusive behaviors are primarily due to personality disorders that can be dismissed.

With intimate partner violence and rape and sexual assault studied as individual concepts, this establishes a blurred line of when sexual violence perpetrated by an intimate partner on another is considered partner violence. There is a narrative of consistent consent in a romantic relationship where the victim may have granted consent at one point, that which becomes permanent consent in a relationship, leading to sexual assault or rape (McKimmie et al., 2020). Victims of sexual violence by strangers or acquaintances are taken more seriously in comparison to those committed by an intimate partner as it is not considered a form of sexual assault, but rather intimacy between partners (McKimme et al., 2020). This contributes to the myth that sexual violence cannot occur between romantic partners.

There are many common fallacies under the concept of victim blaming that constitute as intimate partner violence myths. Revictimization is a component of victim blaming that refers to the cycle of victimization based on the victim's inability to leave their abusive partner. (Baldry & Cinquegrana, 2021). There are many environmental and social characteristics that alter a victim's capability to leave an abusive relationship, such as available resources, the belief in the violence occurring, and experiencing a form of guilt leaving what was once a romantic partner (Baldry & Cinquegrana, 2021). A lack of these factors prevents a victim from being able to protect themselves from the abuse and forces them to continue to remain in that relationship. This has established the myth of revictimization as the victim's fault. Society has placed this burden on victims to be the primary protection for themselves against intimate partner violence therefore if they remain with that abusive partner, they are at fault. Victims that do not leave an abusive

partner are seen as “stupid” or “dumb” for staying in that household or relationship with them as it stems from the belief that leaving that individual will end the violence (Monterrosa, 2021).

Other concepts of victim blaming were introduced by Peters (2008) in the Domestic Violence Myth Acceptance Scale [DVMAS]. This scale has been widely used to evaluate the level of individual beliefs in common societal myths regarding intimate partner violence. Victim blaming is the primary theme of the DVMAS as it includes questions referring to the victim’s position in the environment, such as her psychological characteristics, and her behavior (Peters, 2008). This scale also includes an indicator of severity and glorifies the offender by rationalizing their behavior (Peters, 2008). The DVMAS held various limitations such as the exclusion of multiple genders. Due to certain weaknesses, the Acceptance of Myths About Intimate Partner Violence Against Women Scale [AMIVAW] was created to explore other established intimate partner violence myths that the DVMAS failed to address (Megias et al., 2018). This scale was more inclusive in evaluating different IPV myths in society that go beyond victim blaming. This study found there was a substantial level of the belief of intimate partner violence myths that continue in our society today (Megias et al., 2018).

Intimate partner violence research has closely followed the victimization of women, excluding male victims. Victims of intimate partner violence are believed to be the “battered woman” stereotype who is vulnerable, helpless, and naïve (Monterrosa, 2021, p.9155). The societal interpretation of intimate partner violence victim and offender characteristics has relied heavily on the idea that only women are victims of IPV and men cannot be. This idea that men cannot be victims of IPV is based on the primary idea of masculinity, that can protect themselves against women; therefore, no violence can occur (El Sayed et al. 2022). The gender of the victim and the offender dictates how society will react to the violence that occurs; male-to-female abuse

is understood as more serious and severe in comparison to female-to-male abuse (Wilson & Smirles, 2022). The primary cause behind the outlook on severity based on gender is the differences in physical attributes such as height, strength, and size that create a more fearful response (Wilson & Smirles, 2022). Retrospectively, this demonstrates the foundation for how women are typically not viewed as the aggressor based on their lack of ability.

Male victims experience various forms of dismissal and rejection in society regardless of their sexual orientation. Whether at the hands of another male or female partner, male victims of intimate partner violence have coexisted with myths that label them as weak, abusers themselves, and are met with disbelief (Bates et al., 2019). Male victims also encounter a form of victim blaming as they are viewed to be responsible for their victimization (Thomas & Hart, 2022). This continuance of the female victim-to-male offender myth and that males cannot be victims has prevented male victim support services (Bates et al., 2019). It contributes to the ongoing gender norms that infer women are not physically capable of being aggressive, but they are rather passive, weaker, and need protection. Men are the opposite and are capable of being violent (Bates et al., 2019).

Previous research has closely studied the experience of female victims of intimate partner violence and has failed to address the various types of other genders and relationships that can suffer from this type of violence. The growing research on intimate partner violence has found that gay, bisexual, and other homosexual male relationships are victims of intimate partner violence at higher rates in comparison to cisgender men in heterosexual relationships (Stephenson et al., 2022). This research has noted that “typical” stressors of intimate partner violence, such as financial issues, substance misuse, etc. are prevalent among homosexual male relationships. However, these individuals face additional concerns such as the failure to disclose

one's sexual identity to family or friends, a form of sexual orientation-based discrimination in society, and threats to one's masculinity in addition to intimate partner violence (Stephenson et al., 2022). These findings have defined the higher rates of intimate partner violence that occur in male homosexual relationships that is in comparison to others.

Past and current research has concluded that intimate partner violence demonstrates themes of ongoing gender norms and stereotypes that provide a causality for the violence. Although research on this topic heavily focuses on the male offender to the female victim, it is important to evaluate the environment this violence occurs. A recent study (e.g., Rollero, 2020) concluded that men connected their normalization of violence against their partners to societal gender norms and the stereotypes that men are accustomed to. It is the ideology of male dominance and authority over women that allow men, and society to normalize violence against women (Rollero, 2020). Again, it is the continuum of gendered societal roles where women are seen as weaker and gentle while men are seen as more aggressive and powerful (Bates et al., 2019). Societal beliefs laid the foundation for intimate partner violence myths to be sustained.

Theoretical Framework

George Gerbner's 1998 Cultivation theory describes the concept of the "mean world syndrome" that infers when individuals in society are exposed to media platforms consistently, they are likely to develop reflective opinions and beliefs (Gerbner, 1998). Individuals will cultivate their beliefs to coincide with those the media produces, where one's reality is built off the "media reality" (Gavin & Kruis, 2022, p. 182). Personal social constructs internalize those of the media that demonstrate the relation between violent media and an acceptance of violence. In relation to gender norms and stereotypes, the media's production of intimate partner violence is in a strict lens that encourages IPV myths and only displays incidents of extreme physical harm

and neglects to acknowledge the various other forms of IPV (Rollero, 2019). In the neglection of the different occurrences of IPV, strengthens the myths such as males cannot be victims, IPV is only physical harm, etc.

Social cognitive theory provides an additional understanding of the influence violent media has on societal perceptions and beliefs. The foundation for social cognitive theory lies in the idea that one's behavior, thoughts, and beliefs are reflective of what they are exposed to (Toplu-Demirtas et al., 2020). It focuses on the observations of individuals that conditions their personal attitudes and opinions; therefore, those who are consistently exposed to violent programs within the media idolize these perceptions and apply them to their own reality. Although social cognitive theory provides a primary explanation of the exposure to interpersonal violence in family, this theory provides an alternative explanation for the media's ability to influence an individual's perceptions.

Hypotheses

1. Students that consume high levels of media sources will demonstrate a higher belief in intimate partner violence myths.
2. Students that consume violent media will be more accepting of intimate partner violence myths.

Methodology

Sample and Dataset

This study used a non-probability, availability sampling design. The data was collected from 79 undergraduate students at Cabrini University. These surveys were approved by Cabrini University's Institutional Review Board (IRB) and were collected in the Spring semester of

2023. Professors of various courses at Cabrini University were previously contacted requesting their permission to distribute the survey link to students within their classes. This study's electronic survey was created utilizing Google Forms. Professors that granted their permission were emailed the link to the Google Form to share with their students. This omnibus survey contained 62 questions and a digital consent and warning form that provided researchers' contact information with counseling resources provided.

A total of seventy-nine surveys were collected (*N*=79) and only a small amount of missing data occurred. Within the sample, 73.4% were female (*n*=58), 25.3% were male (*n*=20), and 1.3% selected cisgender (*n*=1). The sample's racial demographics consisted of 76.9% (*n*=60) White participants, 15.4% (*n*=12) Black or African American, 5.1% (*n*=4) Hispanic, Latino, or of Spanish origin, 1.3% (*n*=1) mixed, and 1.3% (*n*=1) White/Asian. Regarding class year, 31.6% (*n*=25) of participants were Juniors, 26.6% (I=21) indicated that they were Freshman, 22.8% (*n*=18) selected Senior, and 19.0% (*n*=15) chose Sophomore. 55.1% (*n*=43) respondents indicated they were a collegiate athlete while 44.9% (*n*=35) were not. The majority of respondents indicated they were 19 (26.6%; *n*=21) or 21 years old (31.6%; *n*=25). See Table 1 for additional demographic statistics of the sample.

Table 1

Sample Demographics

Variable	***Frequency***	***Valid Percent***
Age		
18	11	13.9
19	21	26.6
20	12	15.2
21	25	31.6
22	7	8.9

23	1	1.3
Grade		
Freshman	21	26.6
Sophomore	15	19.0
Junior	25	31.6
Senior	18	22.8
Race/Ethnicity		
White/Caucasian	60	76.9
Black or African American	12	15.4
Hispanic, Latino, or of Spanish origin	4	5.1
Mixed	1	1.3
White/Asian	1	1.3
Gender		
Male	20	25.3
Female	58	73.4
Cisgender	1	1.3
Athletic Participation		
Yes	43	55.1
No	35	44.9

Independent Variables: Media Consumption & Media Violence

The first independent variable for this study is the consumption of media conceptualized through television, online, and news-related media. Within the survey, respondents were asked to indicate the number of hours per day they watch television, spend online, such as social media, and consuming news-related media: On average, how many hours per day do you watch television? On average, how many hours per day are you online (such as social media or using the internet on a computer or cell phone)? On average, how many hours per day do you spend watching the news on television or online or reading news articles? 1=0-1 hour per day, 2=2-4 hours per day, 3=5-6 hours per day, and 4=6 or more hours per day (Felix et al., 2020). 50.6%

(*n*=40) responded 2-4 hours per day of both watching television, and being online, while 84.8% (*n*=67) selected 0-1 hour per day of consuming news-related media.

The second independent variable of this study was the consumption of violent programs or themes within the media. In this survey, participants were asked to indicate the number of times per week they were exposed to or consumed violent programs in the media: How often in the last week were you exposed to programs that contained violence or violent themes? 1=0 times in the last week, 2=1-2 times in the last week, 3=3-4 times in the last week, 4=5-6 times in the last week, and 5=7 or more times in the last week (Holmgren et al., 2019). In total, 43.6% (*n*=34) indicated 1-2 times in the last week, and 28.2% (*n*=22) chose 3-4 times in the last week. See Table 2 for additional media consumption statistics.

Table 2

Media Consumption Statistics

Variable	***Frequency***	***Valid Percent***
Television Hours		
0-1 hour per day	35	44.3
2-4 hours per day	40	50.6
5-6 hours per day	4	5.1
Online Hours		
2-4 hours per day	40	50.6
5-6 hours per day	32	40.5
6 or more hours per day	7	8.9
News Hours		
0-1 hour per day	67	85.9
2-4 hours per day	8	10.3
5-6 hours per day	2	2.6

Violent Media		
0 times in the last week	7	9.0
1-2 times in the last week	34	43.6
3-4 times in the last week	22	28.2
5-6 times in the last week	9	11.5
7 or more times in the last week	6	7.7

Dependent Variable: Beliefs of Intimate Partner Violence Myths

The dependent variable for this study is the belief in intimate partner violence (IPV). Myths regarding IPV can be conceptualized by any well-known misconceptions regarding victims, incidents, perpetrators, etc. To measure students' belief in IPV myths, participants were asked a total of 16 questions. All 16 questions utilized Likert-style questions that ranged from *1*= Strongly Disagree to *5*=Strongly Agree. These questions asked participants to select their level of agreement with various existing popular intimate partner violence myths included in previous studies. For example, the first question asked participants to indicate their agreement with this statement: Intimate Partner Violence does not affect many people (Peters, 2003), on a scale of *1 (strongly disagree)* to *5 (strongly agree)*. The second question asked participants to respond with their level of agreement with this statement: When a man is violent, it is because he lost control of his temper, and it is the victim's fault for provoking him (Peters, 2003) on a scale of *1 (strongly disagree)* to *5 (strongly agree)*. As another example, the ninth question asked participants to indicate their level of agreement with this statement: Men cannot be victims of intimate partner violence (Gover, 2011), on a scale of *1 (strongly disagree)* to *5 (strongly agree)*.

Table 3

Variable Descriptive Statistics

Variable	*N*	*Mean*	*Minimum*	*Maximum*	*Standard Deviation*
Media Consumption					
Television	79	1.61	1	3	.587
Online	79	2.58	2	4	.653
News	78	1.19	1	4	.536
Violent Program/Themes Consumption	78	2.65	1	5	1.055
IPV Scale	75	35.8933	25.00	28.00	6.16348
Age	79	-	18	24	-
Gender	79	-	1	5	-
Race	78	-	1	7	-
Grade	79	-	1	4	-
Athletic Participation	78	-	1	2	-

Results

Media Consumption and Violent Media and Belief in IPV Myth Scale

An additive scale was created to combine the 16 Likert-scale questions to create a belief in myths variable. The creation of the additive scale was appropriate because Cronbach's alpha was .728, which supported the reliability of the scale. The belief in myths scale ranged from 25 to 58, with 25 indicating a low belief in myths and 58 representing a high belief. The most frequent ranking of the beliefs in myths was 32 (10.7%; *n*=8). The mean for the belief in myths

scale was 35.89, which means there was a moderate disagreement in myths of intimate partner violence. The median of the beliefs in myths was 35 (2.7%; *n*=2). See Table 3 for the variable descriptive statistics. See *Figure 1* for the distribution of belief in myth scores.

Figure 1

Distribution of belief in myths levels by college students

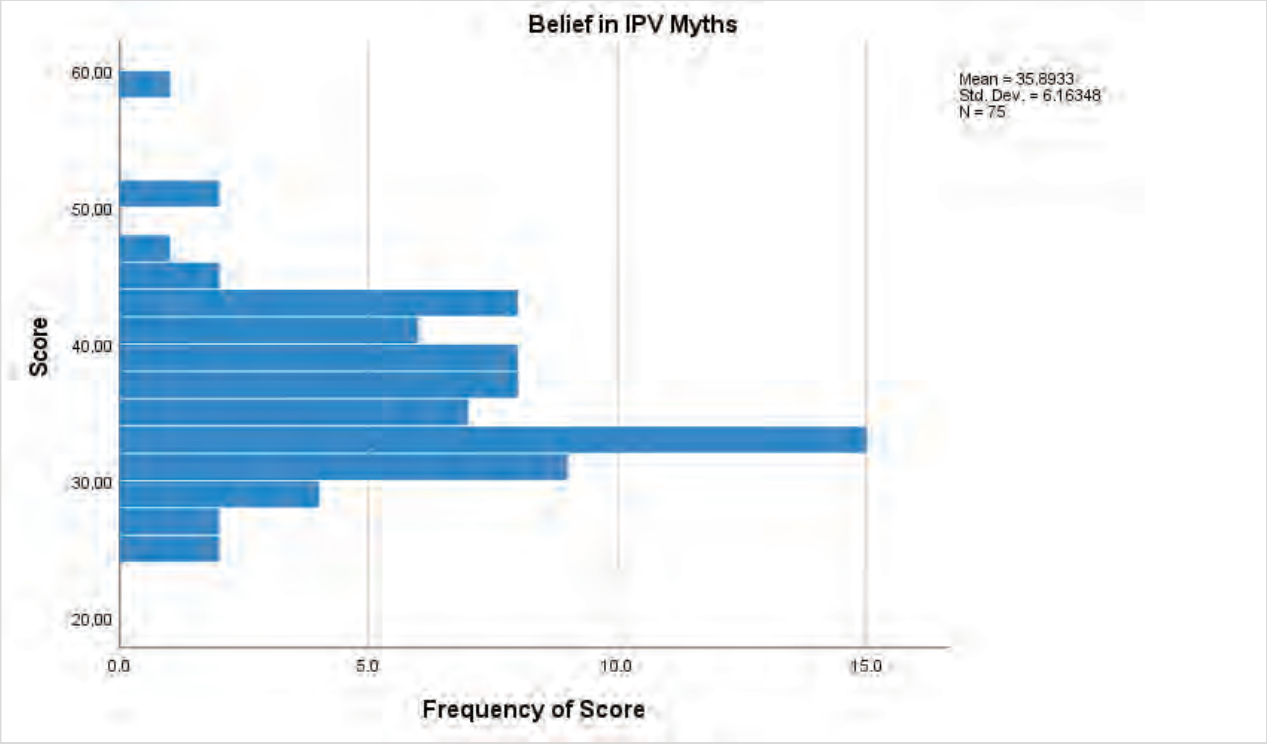

Table 4 showcases the results of the spearmen correlation that was conducted to determine the correlation between the consumption of media and violent media and the belief in intimate partner violence myths. At the conclusion of the Spearmen correlation test, there were no significant relationships found between the variables. Spearmen's rank correlation was computed to assess the first relationship between television consumption and an acceptance of IPV myths. There was no correlation found between the variables of tv consumption and an acceptance of intimate partner violence myths, $r_s(73)=.068$, p=.560. Additionally, spearmen's rank correlation was calculated to evaluate the relationship between the variables of online hours

and a belief in intimate partner violence myths. There was no correlation found between the variables of hours spent online and a belief in IPV myths, $r_s(73)=.058$, p=.619. Spearmen's rank was utilized to determine a relationship between watching the news, or news-related programs and tolerance of intimate partner violence myths. There was no correlation found between the variables of watching the news to being tolerant of IPV myths, $r_s(72)=-.142$, p=.228. Lastly, Spearman's rank correlation was conducted to investigate a relationship between consuming violent media and being in agreement with IPV myths. There was no correlation found between the variables of consuming violent media and demonstrating an agreement with IPV myths, $r_s(73)=.077$, p=.510.

Table 4

Spearman Correlation Media Consumption and IPV Scale

Variables	*(1)*	*(2)*	*(3)*	*(4)*	*(5)*
1) IPV Scale	-	-	-	-	-
	-	-	-	-	-
	.	-	-	-	-
2) Television	.068	-	-	-	-
	.560	-	-	-	-
	75	.	-	-	-
3) Online	.058	.021	-	-	-
	.619	.854	-	-	-
	75	79	.	-	-
4) News	-.142	.032	.405**	-	-
	.228	.782	.000	-	-
	74	78	78	.	-
5) Violent	.077	.176	.071	.048	-
Programs	.510	.123	.535	.677	-
	75	78	78	77	.

Discussion

This study sought to evaluate the relationship between media consumption and the belief in myths relating to intimate partner violence. It also examined an association between violent programs within the media and an acceptance of IPV myths. There was little research done previously regarding this specific topic however, existing literature regarding media consumption, media violence, and intimate partner violence suggested a potential connection between these variables that drove this study (Gavin & Kruis, 2022). With this, it was first hypothesized that students who consume high levels of the media would demonstrate a higher belief in intimate partner violence myths. The results of the spearmen correlation did not support the first hypothesis. and did not indicate a relationship between the variables. The second hypothesis anticipated that students who consume high levels of violent programs within the media would admit a higher acceptance of IPV myths. The results of the spearmen correlation did not support the second hypothesis. These findings do not reflect the expectations of previous literature, as there were no relationships found.

55.1% of the sample participated in a collegiate sport at Cabrini University (*n*=43), while 44.9% (*n*=35) were not. In relation to the media consumption variable, the majority of participants indicated a low level of 2-4 hours per day for television (50.6%; *n*=40) and online hours (50.6%; *n*=40), 85.9% (*n*=67) consume 0-1 hour per day of the news, and 43.6% (*n*=34) consumed violent programs within the last week. These lower levels of media consumption are concurrent with the high percentage of the sample that identified as collegiate athletes. Collegiate athletic participation demands a student's time, energy, and effort which provides an explanation for the lower media consumption hours that were reported. Additionally, athletes as a cohort of students at Cabrini University may have been exposed to a type of training or education that may

have been opposing to the media's potential influence. Outside of athletic participation, low levels of media consumption can be understood as a product of being a student, with classwork and various other activities time may be dedicated to that provides an additional reason for the media consumption findings.

Furthermore, 73.4% (*n*=58) of this study identified as female while only 25.3% (*n*=43) identified as male. Previous research has understood men to be more likely to condemn and support myths compared to women related to IPV based on existing misogynist views (Rollero & De Piccoli, 2020). With the sample size consisting of more female than male students, this may have introduced the lack of acceptance in IPV myths. Misogyny is an ongoing issue that although can exist on all college campuses, there is significantly more popular misogynist-led beliefs on Division I campuses in comparison to Division III (Carey et al., 2022). A significant indicator in the presence of campus sexism is campus involvement, with a recognition of these issues and dedication of resources towards preventing these said views or treatment. Campus involvement tends to be prioritized more among Division II and III universities compared to Division I which does not let issues related to misogyny and sexism go unnoticed (Carey et al., 2022). Cabrini University, as a Division III school, reflects the current literature with a mission of social justice and no tolerance of these beliefs that were evident in the results of this current study.

Limitations, Future Research, & Implications

There were various limitations to this study that may be a contributing factor to the lack of correlation between variables. This study utilized a non-probability availability sampling method with only a sample size of 79 participants between the class years of Freshman (1st Year) to Seniors. The low sample size of this study prevented the sample from being representative of

the entire population in question; therefore, a higher sample size would allow for more generalizability. Certain factors that contributed to the small sample size were identified as the time frame in which the survey was distributed, as it did not provide ample time to receive the necessary number of responses. The digital format of the electronic survey, although convenient, may have also limited the number of responses as a hard copy distributed in class would have resulted in a higher response rate based on the demand. title of multiple topics, that included myths of intimate partner violence can be seen as a factor in students not interested in responding to questions related to IPV. Additionally, the extended length of the survey of 62 questions could have prevented students from being motivated to complete it. The

Future research on this topic should be conducted to further evaluate if there is a relationship between media consumption and violent media and the belief in intimate partner violence. Future research should expand on this current study as it would be interesting to limit the media consumption variable to only one type of media platform, such as social media, to evaluate the relationship with the belief in IPV myths. Moreover, it would be interesting to see if one's gender or major has a direct impact on their belief in myths related to intimate partner violence. Future research may also consider limiting the number of IPV myths presented in a survey and only introducing IPV myths under a certain perspective, such as victim blaming or characteristics of victims and offenders.

Despite the lack of a statistical correlation, this current study adds to the existing literature relating to this topic. Previous research assumed a relationship among these variables that this study evaluated; therefore, future research can expand on the lack of relationship found to discover another influential variable. It is imperative for future research to discover what factors may contribute to the belief in IPV myths. This current study may be beneficial to be

conducted elsewhere outside of a Division III liberal arts college to gain additional insight into college students or a separate population.

Conclusion

Overall, the goal of this current study was to evaluate the relationship between a college student's level of media consumption and the belief in intimate partner violence myths. Based on the results of this study, there is not a significant relationship between the 4 independent variables and the 16 dependent variables on of IPV scale. However, the topic of this study adds to the current body of literature as it suggests the influence of individual perspectives on intimate partner violence exists outside of media platforms. It guides future research to look at factors outside of media consumption that encourage the continuation of IPV myths in society that may not be expected. Discovering this influential figure is an important piece to ending the stigmatization and lack of awareness behind all occurrences of intimate partner violence. This study on media consumption is the beginning of future research on this topic.

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El voto femenino y los derechos de las mujeres después de la Guerra Civil Española

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El voto femenino y los derechos de las mujeres después de la Guerra Civil Española

Entre la época de la Segunda República Española y la época posterior a la Guerra Civil española, las mujeres de España experimentaron dos extremos polarizadores de liberación y opresión. En la Segunda República las mujeres empezaron a recibir el mismo trato que los hombres debido a un cambio en las leyes y ganaron su derecho al voto, sin embargo, su liberación se acabó rápidamente después de la guerra civil ya que las mujeres se vieron obligadas a ajustarse a las nuevas normas de la sociedad. Estas nuevas normas oprimían a las mujeres porque las presionaban para que se convirtieran en amas de casa y tuvieran hijos. Además, las leyes injustas creadas después de la guerra civil restringían la participación de las mujeres en la vida pública.

Antes de la proclamación de la Segunda República Española el 14 de abril de 1931, había una división extrema entre los ricos y los pobres; toda la tierra era la propiedad de un pequeño grupo de personas ricas, la tasa de desempleo era alta y muchas personas eran analfabetas. Esto dividió al país en dos partidos. La izquierda estaba compuesta por los trabajadores urbanos y agrícolas y la clase media educada que exigía salarios estables, reforma agraria y separación de la iglesia y el estado. En cambio, la derecha estaba formada por católicos, terratenientes y empresarios que exigía proteger su riqueza, sus tierras, la iglesia y la restauración de la monarquía. Debido a la crisis económica y educativa y a las crecientes tensiones políticas, se celebraron las elecciones municipales que resultaron en la victoria del partido republicano, la proclamación de la Segunda República Española y la renuncia del rey Alfonso XIII que fue sustituido por Manuel Azaña. Las Cortes Constituyentes fueron convocadas en agosto de 1931 para redactar específicamente una nueva constitución democrática y progresista que otorgaría a

las mujeres nuevas libertades y derechos por primera vez en la historia de España (Eco Republicano, 2015).

Algunos de los aspectos más relevantes para las mujeres en la nueva constitución fueron la igualdad ante la ley, no discriminación por origen, sexo o riqueza, el derecho a ser elegidas para cargos públicos sin restricciones y el sufragio universal que permitía a las mujeres el derecho al voto (Eco Republicano, 2015). Sin embargo, el voto femenino no fue fácil de obtener porque creó un complejo debate entre los miembros de las Cortes Constituyentes. En particular, Victoria Kent y Clara Campoamor mantuvieron posiciones opuestas sobre el voto femenino, aunque ambas abogaron por el avance de la condición social de la mujer en España. Victoria Kent, miembro del Partido Republicano Radical Socialista, fue una de las primeras mujeres en ejercer la abogacía en España y fue la primera mujer en ocupar la Dirección General en España en la que promovió reformas humanitarias y laicas en la vida carcelaria. Kent argumentó que el voto femenino debía posponerse porque creía que si las mujeres obtenían el derecho al voto, la mayoría de las mujeres votarían al partido nacionalista y causarían que el partido republicano perdiera su poder. (Arias, 2017). Explicó, “Pido a la Cámara que despierte la conciencia republicana, que avive la fe liberal y democrática y que aplace el voto para la mujer...no es cuestión de capacidad; es cuestión de oportunidad para la República.” (Arias, 2017 P. 40) Kent pensaba que la mejor opción era darle el voto a las mujeres cuando ellas tuviesen la educación política suficiente para votar responsablemente. Clara Campoamor, miembro del Partido Radical Republicano, que era también una abogada, escritora e influyente promotora del sufragio femenino. En contraste con las ideas de Kent, Campoamor argumentó que no era apropiado retrasar el reconocimiento de un derecho con el pretexto de que podría ser abusado y que las mujeres deberían tener el derecho al voto inmediatamente. Por último, las mujeres de España

ganaron su derecho al voto con 161 votos a favor y 121 en contra. Utilizaron su nuevo derecho al voto en las elecciones de 1933 en las que ganó la derecha. Aunque una coalición de derechas ganó, no hay ninguna referencia directa al impacto del voto femenino en los resultados de las elecciones. Por lo tanto, es difícil decir si las dudas de Kent eran lógicas (Arias, 2017).

Con el paso de los años, las tensiones políticas siguieron creciendo entre republicanos y nacionalistas. Esta división en el país eventualmente condujo a La Guerra Civil española que comenzó el 17 de julio de 1936, cuando los generales nacionalistas Emilio Mola y Francisco Franco intentaron un golpe de estado y una rebelión militar con la intención de derrocar al gobierno del Frente Popular, una coalición de izquierdas (United States Holocaust Memorial Museum, n.d.). Las mujeres de ambos lados políticos tuvieron un papel importante en la guerra civil. Fueron consideradas colaboradoras de pleno derecho en el esfuerzo bélico en el bando nacionalista, pero especialmente en el bando republicano. Se animó a las mujeres a unirse a la guerra y a trabajar con los hombres en las fábricas. Mientras que las mujeres del bando nacionalista participaron activamente en grupos de ayuda y bienestar. (Films Media Group, 1993). Este fue un logro y un avance muy importante para las mujeres porque por primera vez después de la Segunda República, las mujeres de España eran participantes activas en la vida pública.

La guerra civil española terminó en 1939 con la victoria de los nacionalistas. Después de la guerra civil, la dictadura de Francisco Franco borró todo el trabajo que las coaliciones de la izquierda había establecido para ayudar al avance de las mujeres con sus libertades y derechos. Las nuevas normas para las mujeres creadas bajo la dictadura se impartían a través del Nodo y de la iglesia. La Sección Femenina se creó con el objetivo de formar a las mujeres dentro de la moral falangista, una ideología política fascista que enfatizaba la autoridad de la iglesia católica,

las ideas conservadoras y los roles de género extremos para hombres y mujeres. Prepararon a las mujeres para tener familias, seguir la religión, específicamente el catolicismo y tener un fuerte sentido del nacionalismo y orgullo por España. Consideraban que la mayor misión en la vida de la mujer era ser madre para aumentar la población de patriotas y cristianos debido a las numerosas pérdidas sufridas durante la guerra civil. En la Sección Femenina, existía la escuela-hogar para enseñar a las mujeres cómo hacer las tareas del hogar y convertirse en el ama de casa perfecta e ideal. Por ejemplo, las mujeres estudiaban economía doméstica y cómo lavar, limpiar, planchar y coser (Andrés, 2021). A pesar de que las mujeres aprendían a ser madres, a las mujeres se les seguía diciendo que todavía eran inferiores a sus maridos, "Por otro lado, a pesar de que la mujer se dedicaba fundamentalmente al cuidado de los hijos, se creía que incluso en ese ámbito el padre era legítimamente superior." (Andrés, 2021) Las nuevas normas para las mujeres restringían sus libertades y les hacían creer que su único destino era casarse con un hombre, tener hijos y convertirse en una esclava de la casa.

El avance de las mujeres no sólo estaba restringido por las normas de la sociedad, sino también por la ley. En la década de 1940, había barreras que limitaban el acceso de las mujeres al trabajo, "Las empresas podían despedir a la mujer una vez contrajese matrimonio. Además, de necesitar la autorización de su marido para poder firmar cualquier contrato." (Andrés, 2021) Las mujeres no podían ser magistradas, fiscales o juezas en la administración pública hasta 1966 y las mujeres no podían ser agentes de policía ni formar parte de las fuerzas armadas hasta 1975. También, cuando una mujer se casaba, no podía abrir su propia cuenta de banco sin el permiso y la autorización de su marido (Andrés, 2021). Esta era una diferencia muy drástica con respecto a los varios derechos y capacidades que las mujeres tenían bajo la constitución republicana de 1931. Además, se crearon leyes que afectaban al cuerpo de las mujeres y a las relaciones

matrimoniales. Por ejemplo, una persona era castigada con prisión por facilitar un aborto. Sin embargo, si una mujer tenía un aborto para proteger a su familia de la vergüenza y/o si la mujer estaba soltera, el castigo era menor para ella (Andrés, 2021). Las leyes sobre el aborto estaban más preocupadas por las apariencias de la mujer y su familia que por los deseos y el bienestar de la mujer. Además, las mujeres y los hombres eran castigados de formas diferentes por el adulterio, "Para la mujer, el adulterio era castigado con dureza…para castigarse con penas de prisión, mientras que para que el esposo recibiese una pena equiparable, era necesario que este acto se realizara en el hogar familiar o notablemente fuera de él." (Andrés, 2021) Es evidente que estas leyes y procedimientos fueron promulgados para oprimir a las mujeres, mantenerlas sumisas a los hombres y controlar sus cuerpos.

En conclusión, bajo el gobierno republicano, las mujeres pudieron realizar avances significativos e importantes en su liberación a través de la obtención del derecho al voto, la igualdad ante la ley y la capacidad de trabajar en empleos de hombres durante la guerra civil. Al contrario, después de la guerra civil, bajo una dictadura fascista, las mujeres fueron transformadas en esclavas de la casa y perdieron completamente su autonomía a través de leyes desiguales e injustas que restringían su acceso a participar en la vida pública.

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D**evelopment of a flow cytometry method to investigate poly (I:C) stimulation in coelomocytes of *Eisenia hortensis***

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Abstract
The main goal of this study was to investigate the cellular effects of polyinosinic-polycytidylic acid (poly (I:C)) in the earthworm *Eisenia hortensis*. Poly (I:C) is a double-stranded RNA mimetic that also acts as a Toll-like receptor 3 (TLR3) agonist, known to be involved in stimulating antiviral interferon and pro-inflammatory responses of innate immunity. This stimulant has also been used to study innate immunity in other invertebrates, including the Pacific oyster *Crassostrea gigas*, and the Chinese mitten crab *Eriocheir sinensis*. Coelomocytes, the white blood cell equivalents in *E. hortensis*, were extracted, cultured with poly (I:C), and treated with dihydroethidium (DHE) to determine if pro-inflammatory responses are induced in vitro by measuring reactive oxygen species (ROS) production using flow cytometry. DHE is a fluorescent marker used for the detection of hydrogen peroxide, a molecular indicator of intracellular pro-inflammatory events. Using gating techniques, analyses were confined to primarily to the hyaline amoebocyte subpopulation in order to exclude the autofluorescent chloragocytes. Fluorescence was measured using the FL-1 detector of the flow cytometer and data was analyzed using WinList software and Microsoft Excel. Statistically significant results, as determined by the student t-test, were obtained in two independent assays for two of the three coelomocyte populations tested in each assay. These preliminary results suggest that poly (I:C) has the capacity to increase pro-inflammatory immune responses in coelomocytes of *E. hortensis*, as shown by higher levels of ROS production. In future studies, it would be interesting to analyze the effects of poly (I:C) on phagocytosis, and also examine the signaling pathways involved in the innate immune response of this invertebrate species to the TLR3 agonist used in this study.

Introduction

The ability of an organism to fight off the ingress of a foreign agent depends on two key factors, the innate and the adaptive immune responses. While both of these types of immunity are crucially important during an infection, it is important to understand the differences between them. Innate immunity is present in both vertebrates and invertebrates, and is characterized as the first line of defense, which as the name suggests, it is the one that kicks in almost instantly once a pathogen finds its way into healthy cells (Wang & He, 2019). This type of immunity is classified as non-specific because it protects against any pathogen expressing evolutionarily conserved components (patterns) without discrimination or specificity. Moreover, it relies on the recognition of foreign molecules through the use of pattern recognition receptors (PRRs) that are germline encoded. Lastly, innate immunity is incapable of developing memory, and therefore,

upon secondary encounter of the same pathogenic agent, the innate response will be similar to the first one (Vivier & Malissen, 2005).

While innate immunity is the first line of defense when an infection is under way, adaptive immunity also plays a key role in this process. Adaptive immunity can only be found in vertebrates, it is specific, it takes days to weeks to get formed and then clear an infection and it has immunologic memory (Wang & He, 2019). In addition to these characteristics, the adaptive response depends on the correct binding of antigen receptors (generated through site-specific somatic recombination) to the foreign antigens (Vivier & Malissen, 2005). One more noteworthy feature of adaptive immunity includes the function of B and T lymphocytes. B cells mature in the bone marrow and are empowered with the ability to produce antibodies necessary for binding to invading antigens with high specificity in order to target them for elimination. T cells, however, mature in the thymus, and are mostly associated with cytotoxic effects and helping other cells to kill the pathogen (Bonilla & Oettgen, 2010).

Pathogens express certain pathogen associated molecular patterns (PAMPs) that help them with their survival. Examples of PAMPs include lipopolysaccharide (LPS), peptidoglycan, and flagellin found in bacterial cells, zymosan that is part of fungal cells, and viral RNA (e.g. single- and double-stranded RNA) found in viruses (Tang et al., 2012). When these foreign agents enter an organism, PRRs recognize and bind to them, with the purpose of stimulating pro-inflammatory cytokines and type I interferon (IFN) production, whose role is to alert other cells about an ongoing infection, in order for them to prepare accordingly to fight it off. Furthermore, type I IFN helps facilitate the expression of interferon stimulated genes (ISGs) that serve as a shield for neighboring cells in close proximity to the infected one during an infection (Wang & He, 2019).

PRRs vary in their cellular location as they are found on the plasma membrane, in the cytoplasm, or on the membranes of endolysosomal vesicles. These are divided into five groups; toll-like receptors (TLRs), which are found in immune cells, retinoic acid-inducible gene-I-like receptors (RLRs), Nod-like receptors (NLRs), C-type lectin receptors (CLRs), and stimulator of interferon genes (STING). TLRs are found in the plasma membrane with ligands such as flagellin and LPS, or in the endolysosome with ligands including single- or double-stranded RNA. RLRs, however, are only located in the cytoplasm, and they express ligands such as short or long double-stranded RNA. Similarly, NLRs are another subgroup of PRRs which can only be detected in the cytoplasm with g-D-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP) for ligands. The CLRs, on the other hand are only found in the plasma membrane and express ligands such as β-glucan or spliceosome-associated protein 130 (SAP130) (Takeuchi & Akira, 2010). In 2008, another PRR was discovered, and it was named STING (also referred to as MITA or ERIS). This PRR resides in the cytoplasm and its ligands include bacteria-derived cyclic di-guanylate monophosphate (c-dGMP) or cyclic di-adenosine monophosphate (c-dAMP) (Wan et al., 2020).

In the present study, the potential involvement of TLR3 in recognition of poly (I:C) in immune cells of the earthworm *Eisenia hortensis* (the European night crawler) is addressed indirectly by evaluating production of reactive oxygen species (ROS). This TLR subgroup is located in the endosome and serves to recognize viral nucleic acids. Upon successful sensitization of TLR3 with the viral double-stranded RNA (the ligand), Toll/Interleukin-1 receptors (TIRs) bind to adaptor proteins. Depending on the adaptor proteins, one of the two signaling pathways may proceed; the MyD88-dependent or the MyD88-independent pathway. The first one has the potential to lead to the generation of helpful pro-inflammatory cytokines,

including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), and chemokines. After a series of kinase cascades and ubiquitination, nuclear factor kappa B (NFκB) is translocated to the nucleus, where it regulates the transcription of pro-inflammatory genes. These gene products affect how the immune system responds to pathogenic stimuli in order to eliminate the infection (Li & Wu, 2021). Apart from NFκB activation, TLR3 also influences the production of type I interferon (IFN), which is a crucial component of inflammation. Additionally, a study revealed that ROS generated by the activity of TLR3 amplify the production and release of pro-inflammatory mediators and are necessary for the activation of NFκB. All of these components are crucial for the inflammatory response, which finally culminates in the production of type I IFN (Yang et al., 2013).

Although nucleic acid sensing seems like a simple process, there is one more step that needs to be taken into consideration when referring to this concept, and that is the capacity of cells to distinguish between self and non-self components. One way in which this can happen is through methylation of nucleic acids. Methylation enables RIG-I to distinguish self from non-self through the addition of a 7-methylguanosine cap at the 5' ends of self mRNAs (Wang & He, 2019). TLR9, in contrast, can spot non-self DNA through CpG motifs. Since CpG motifs are normally heavily methylated in mammalian cells, they can easily be detected and differentiated from those of bacterial cells that do not possess CpG motifs with a great degree of methylation (Kawasaki & Kawai, 2019).

One way in which innate immunity can respond to and eliminate an infection is through phagocytosis. This important cellular process requires a receptor to bind to a target ligand, in order for it to proceed. Its effects are elicited when phagocytes (such as neutrophils and macrophages) get recruited to the site of infection, engulf the foreign agent to form the

phagosome that fuses with a lysosome to form the phagolysosome, and destroys the ingested material through the release of peroxidases and degradative lysosomal enzymes, ROS, and reactive nitrogen intermediates (Uribe-Querol & Rosales, 2020). Despite the fact that phagocytosis is mostly known as an innate immunity response, since it can break down bacteria, viruses, and fungal cells causing infection in an organism, it also has the capacity to help with the presentation of antigens to lymphocytes in adaptive immunity (Lim et al., 2017).

A study conducted by Kokhanyuk et al. (2021) aimed to evaluate whether human THP-1 cells (human monocytic leukemia cell line) and coelomocytes of the earthworm, *Eisenia andrei*, exhibit similarities in terms of phagocytosis. In order to do so, the scientists cultured THP-1 cells, and allowed some of them to differentiate. In the meantime, coelomocytes (earthworm cells) were extruded from *E. andrei*. Then THP-1 cells, THP-1 differentiated cells, and coelomocytes were transferred to 24-well plates, where they were pre-treated with endocytosis inhibitors. Upon completion of this step, the cells were challenged with fluorescein isothiocyanate (FITC)-labeled *Escherichia coli* and *Staphylococcus aureus* in vitro. Trypan blue was also added to the samples, with the purpose of distinguishing bound from ingested bacteria. After flow cytometric analysis of the results, it was found that although signaling pathways present some differences, human THP-1 cells and earthworm coelomocytes engulfed bacteria, thus supporting the proposal that engulfment processes of phagocytosis are highly conserved.

The observation that fewer studies exploring innate immune responses have been conducted in invertebrates, calls for scientists to turn their focus to this area as well, in order to explore the immune responses and defenses utilized by these organisms. The study described in this paper involves the induction of ROS production in coelomocytes of the earthworm *E. hortensis* through the use of poly (I:C). Poly (I:C) is a double-stranded RNA mimetic with the

ability to stimulate the innate immune system through the TLR and RLR families. Additionally, it is a TLR3 agonist, and it induces type I IFNs, thus mimicking antiviral and pro-inflammatory responses (Jang & Song, 2020). This immune stimulant has been used to study immune responses in invertebrates, such as the Pacific oyster *Crassostrea gigas* (Huang et al., 2022), and the Chinese mitten crab *Eriocheir sinensis* (Qi et al., 2019). In the present study, poly (I:C) was utilized with the purpose of inducing ROS production (such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide) in earthworm immune cells. These chemicals can be formed as a result of cellular metabolism, inflammation, or stress. At low levels, ROS are safe for the organism, and they can even serve as signaling pathway assistants, but when they exceed certain levels in the cell, oxidative damage and challenges to cell viability may result (Jakubczyk et al., 2020). The experiments conducted in this study used the fluorescent dye dihydroethidium (DHE) and flow cytometry to monitor ROS production in earthworm coelomocytes. This technology makes use of photodiodes and photomultiplier tubes to detect fluorescent and scattered light signals produced from a single cell suspension, with the purpose of converting those light signals into electronic measurements that are subsequently analyzed using computer software for quantitative measurements (McKinnon, 2018).

Materials and methods

This experiment involved the use of coelomocytes derived from the coelomic cavity of *E. hortensis* to study the induction of ROS production through the action of poly (I:C). There are three types of coelomocytes in *E. hortensis*; eleocytes (also called chloragocytes which are highly autofluorescent), granular amoebocytes (which exhibit phagocytic and NK-like activity), and hyaline amoebocytes (which are highly phagocytic) (Fuller-Espie, 2010). The hyaline

amoebocytes were preferentially analyzed in this study by using gating strategies that permitted exclusion of irrelevant cell types and inclusion of desired cell types during data acquisition.

Animal husbandry

E. hortensis earthworms (also known as European night crawlers) were purchased from Uncle Jim's Worm Farm (2046 Henry Lane, Spring Grove, PA 17362; USDA Permit #P526P-14-01572). All habitat components were autoclaved prior to use. The earthworms were kept in clean containers that were filled with 500mL beaker-filled pine chips, 250mL beaker-filled Hyland Teklad corncob bedding, 250mL beaker-filled water, 1 cup of oatmeal, and baby food liberally spread over surface, and shredded paper towels were added on top, in order to mimic their natural environment (Daly et al., 2017). Changes of their habitats were made every 3-4 days to avoid mold growth and ensure the health of the earthworms.

Preparation of earthworms for extrusion

One day before the extrusion experiment, a pre-determined number of earthworms were set up by thoroughly washing them with deionized water and placing them into Petri dishes containing pieces of paper towel soaked in amphotericin B (FUNGIZONE) (2.5µg/mL). The purpose of this was to help the earthworms empty their gastrointestinal tracts, so that they do not release any secretions during the extrusion of their coelomocytes that might contaminate the cultures, and to reduce risk of fungal contaminants from the earthworm's surface. The plates were kept in the dark overnight at room temperature (Daly et al., 2017).

Extrusion of coelomocytes and preparation of cells for hemocytometer enumeration

For this part of the lab, the extrusion buffer was first prepared by mixing BD FACSFlow sheath fluid with HyClone phosphate buffer saline (PBS) in a 60:40 ratio. Then 3mL of extrusion buffer was added to each trough, and one earthworm was carefully transferred by using sterile forceps

(rinsed with 70% ethanol before use). Once the earthworms extruded their coelomocytes through their dorsal pores, the mixture of buffer and cells was transferred to 15mL conical test tubes. Then 0.25mL of Accumax™ (cell disaggregation solution) was added to each of the tubes utilized, and after mixing, samples were incubated for 2 minutes on ice. Samples were then diluted with 5mL of PBS and the tubes were subjected to a centrifugation at 800 rpm (g = 131 rcf), 4℃, for 5 min (Daly et al., 2017). The supernatants derived were decanted, while the pellets were preserved and flicked slightly before resuspending in 0.5mL of HyClone SFX insect medium supplemented with10% newborn calf serum (Sigma Aldrich), 1X non-essential amino acids (HyClone), 1X glutamine (HyClone), and 1X penicillin, streptomycin, and amphotericin B (Gibco)). After mixing, 8µL of cell sample was transferred to a hemocytometer, placed under a phase contrast microscope, and the number of eleocytes and amoebocytes was determined using 200X magnification. This step was repeated for each coelomocyte sample. Samples with a quality score of 9 or 10 (percentage of autofluorescent eleocytes between 0 and 10%) were selected for each assay when possible. The concentration of cells was adjusted as close to 2×10^6 cells/mL as possible with the goal of using 2×10^5 coelomocytes per test well.

Assay development for ROS detection

Because this methodology had never been reported previously for ROS production in response to poly (I:C) in earthworm coelomocytes, it was important to develop an assay that would provide reliable and reproducible results. Initially, certain parameters were altered (type of dye used, incubation times, and concentration of poly (I:C) to establish criteria needed to capture ROS production. For each one of the assays performed, the cell samples were used in duplicates or triplicates including controls that only received PBS for instrument settings, controls that were mixed with dihydroethidium (DHE) or dihydrorhodamine 123 (DHR123), and experimental

samples receiving a combination treatment of poly (I:C) and DHE. All of the samples were added to wells of a 96-well round-bottom plates. **Table 1** shows the different conditions utilized during assay development. Note that not all data obtained from every assay is included in this paper.

Flow cytometry and WinList analysis

Once the wells were loaded with the samples as described above and incubation took place, flow cytometric analysis followed. The cells were pipetted up and down before transferring them to the flow cytometer tubes with great care, making sure that the room was darkened, in order to avoid interaction of light with the fluorescent dyes (DHE and/or DHR123) to avoid photobleaching of dyes. Then, the samples were run on BD FACSCalibur flow cytometer (using forward scatter (FSC), side scatter (SSC), and fluorescence 1 and 2 (FL-1 and FL-2) parameters for data acquisition). The Listmode data files were transferred to a flash drive for analysis by using Verity WinList 9.0.1 software.

Statistical analysis

The data obtained from both the flow cytometric and the WinList analysis were utilized, so as to run a student's t-test paired two sample for means, using Microsoft Excel. The purpose of this test was to check for the significance of the findings, and whether poly (I:C) stimulated levels of ROS production significantly higher than untreated samples ($p < 0.05$).

Results

This lab experiment was divided into many sections with the purpose of assessing the ability of poly (I:C) to induce ROS production in *E. hortensis* coelomocytes. Once the coelomocytes were extruded through the dorsal pores of the earthworms, they were washed and enumerated with the help of a hemocytometer, so as to select the most suitable samples (those

that exhibited lowest amount of eleocytes and were as close to 2×10^6 cells/mL as possible). After this step, the cells were treated in duplicates (Assay a) or in triplicates (Assay b) with medium (controls), DHE in medium, or DHE plus poly (I:C) in medium and incubated for ~20hrs. The following day, the cells were transferred to flow cytometry tubes, and subjected to flow cytometric analysis using FSC, SSC, FL-1, and FL-2 detectors. Then, the Listmode data files were analyzed through the use of WinList 9.0.1 software. After carefully examining the findings for the first four assays performed, it was concluded that the experimental conditions used for Assay 2, provided the best results, and so this one was carried out one more time under the same experimental conditions, in order to test the reproducibility of the results. Hereafter, Assay 2 is referred to as Assay a, and the assay carried out to reproduce experimental findings is Assay b. In both assays, coelomocytes isolated from three different earthworms were used.

Figure 1 illustrates the different graphs generated after analysis of the data derived from Assay a. Each panel is representative of conditions run in duplicates. **Figure 1A** shows a representative population of cells (R1, highlighted in red) selected for experimental evaluation (4.82%) based on the first three earthworm samples analyzed. **Figures 1B-1D** depict the overlays created for the purpose of comparing the conditions for three different earthworms tested in this experiment, gated on R1. R1 correlates with large coelomocytes (hyaline amoebocytes) based on low autofluorescent and scatter properties a subpopulation of coelomocytes known to exhibit phagocytic activity (Fuller-Espie, 2010). The negative control for autofluorescence that appears in purple in **Figure 1B** was similar for all three of the earthworms used in this assay, and therefore, it was only included in this panel of figure. A second region (R2) based on this negative control provided the threshold for discrimination between DHE-negative versus DHE-positive coelomocytes in each sample during analysis. The FL-2 positive fluorescence events

were 38.42% for the untreated (DHE-only) population of cells (shown in turquoise), and 59.38% for the poly (I:C) treated sample (DHE + poly (I:C)). In **Figure 1C**, the fluorescence events for the second earthworm sample, were 44.75% for the untreated, and 70.74% for the poly (I:C) treated sample. **Figure 1D** shows that the fluorescence events observed for the third untreated earthworm sample were 76.68%, while FL-2 positive events for the poly (I:C) treatment increased to 84.90%.

This experiment was carried out again Assay b to ensure that the results observed in Assay a were reproducible. The same procedure was followed, and the graphs shown in **Figure 2** were acquired. **Figure 2A** depicts the population that was chosen for further analysis (5.80%). The fluorescence events in **Figure 2B** were 7.59% for the control (shown in purple), 25.27% for the untreated sample (shown in turquoise), and 66.16% for the poly (I:C) treated sample (shown in blue). **Figure 2C** indicates that the fluorescence events for this particular earthworm were 21.60%, and they increased significantly in the treatment group, reaching a percentage as high as 70.95%. Lastly, **Figure 2D** shows that the percentage of fluorescent events for the untreated group was 33.93%, and it went up to 65.45% for the poly (I:C) treated sample.

These findings were evaluated for their significance using a student's t-test paired two sample for means in Microsoft Excel. This test was utilized to compare the fluorescence events between the untreated and the poly (I:C) treated samples for each earthworm whose coelomocytes were extruded. If $p < 0.05$, the results were considered to be significant.

Figure 3 shows a bar graph with the y axis indicating the %FL-2 positive events in R2, and the x axis depicting the different treatment conditions used for coelomocytes from three earthworms (EW1-EW3) in Assay a. The student's t-test found that only the results obtained from EW1 and EW3 were statistically significant ($p < 0.05$).

Lastly, **Figure 4** illustrates the bar graph generated for Assay b. After subjecting the results to a student's t-test, it was found that only EW5 and EW6 had statistically significant results ($p < 0.05$). The results indicated that the experiment was reproducible and Assay b validated the results of Assay a.

Discussion

Upon infection with a pathogen, the body relies on the action of innate and adaptive immunity. Despite the differences between these two types of immunity, the ultimate goal of both of them is to activate a signal transduction pathway that will not only alarm the neighboring cells about an ongoing infection, but also eliminate the infectious agent. Although many studies have investigated the adaptive and innate immune responses in vertebrates, very few have focused on invertebrates.

This study aimed to assess the effects of poly (I:C) in coelomocytes of *E. hortensis* by detecting ROS production through the use of a fluorescent marker, DHE, and flow cytometry. The coelomocytes were extruded following an overnight incubation of the earthworms with FUNGIZONE (to empty out their gastrointestinal tracts and minimize contaminants), and then they were washed and enumerated with a hemocytometer. After that, an assay was developed by having controls for instrument setup (coelomocytes only), negative controls (untreated coelomocytes + DHE), and poly (I:C)-treated samples. Four different assays were carried out, as shown in **Table 1**, in order to determine the optimal conditions that yielded the most favorable results (data not shown for all assays). Once it was found that Assay 2 (Assay a) exhibited statistically significant results above control conditions, it was repeated one more time (Assay b) to ensure reproducibility. The final results were analyzed through the use of flow cytometry, WinList, and Microsoft Excel.

In both assays, the hyaline amoebocytes were gated in R1, in order to separate them from the other coelomocyte subpopulations, and to eliminate cellular debris and cell aggregates (**Figures 1A** and **2A**). Histogram overlays were used for the better visualization of the differences in fluorescence intensity between the untreated and the treated coelomocyte samples. The groups shown in purple, in **Figure 1B** and **2B**, were utilized for instrument setup, while the negative control (untreated group) (turquoise) was used to check for background fluorescence. In both assays, the poly (I:C)-treated coelomocytes (blue) exhibited higher percentages for FL-2 positive events, compared to the untreated samples (**Figures 1B-D, 2B-2D**). These findings were further confirmed through analysis on Excel, which additionally indicated that two out of the three earthworm coelomocyte samples used in each case, exhibited statistically significant results ($p < 0.05$) (**Figures 3-4**).

According to these preliminary findings, it was concluded that poly (I:C) has the capacity to induce pro-inflammatory responses *in vitro* and generate ROS in coelomocytes of *E. hortensis*. In future studies, it would be interesting to analyze the effects of poly (I:C) on phagocytosis using fluorescently-labeled bacteria and flow cytometry, as well as to investigate the signaling pathways induced by this immune stimulant in coelomocytes of *E. hortensis*. Finally, it would be useful to verify the involvement of TLR3 in poly (I:C)-induced production of ROS.

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Appendix: Tables and Figures

	Assay 1	Assay 2	Assay 3	Assay 4a	Assay 4b	Assay 5
Volume/well during incubation	200µL	200µL	200µL	200µL	200µL	200µL
#coelomocytes/well	2×10^5 cells	2×10^5 cells	2×10^5 cells	2×10^5 cells	2×10^5 cells	2×10^5 cells
[DHE]	5µM	5µM	5µM	5µM	NA	5µM
[DHR123]	NA	NA	NA	NA	5µM	NA
Poly (I:C)	10µg/mL	100µg/mL	100µg/mL	100µg/mL	100µg/mL	100µg/mL
Incubation period	20 hours	20 hours	5.5 hours	4 hours	4 hours	20 hours

Table 1. *Comparison of conditions used during development of experimental assay.* All of the assays used the same volume/well during incubation and coelomocyte number/well. The concentration of poly (I:C) was increased ten-fold after Assay 1, and Assay 4b used dihydrorhodamine (DHR123 - another fluorescence indicator of ROS production) instead of DHE. Lastly, incubation period varied, to check effects that this parameter had on the final results.

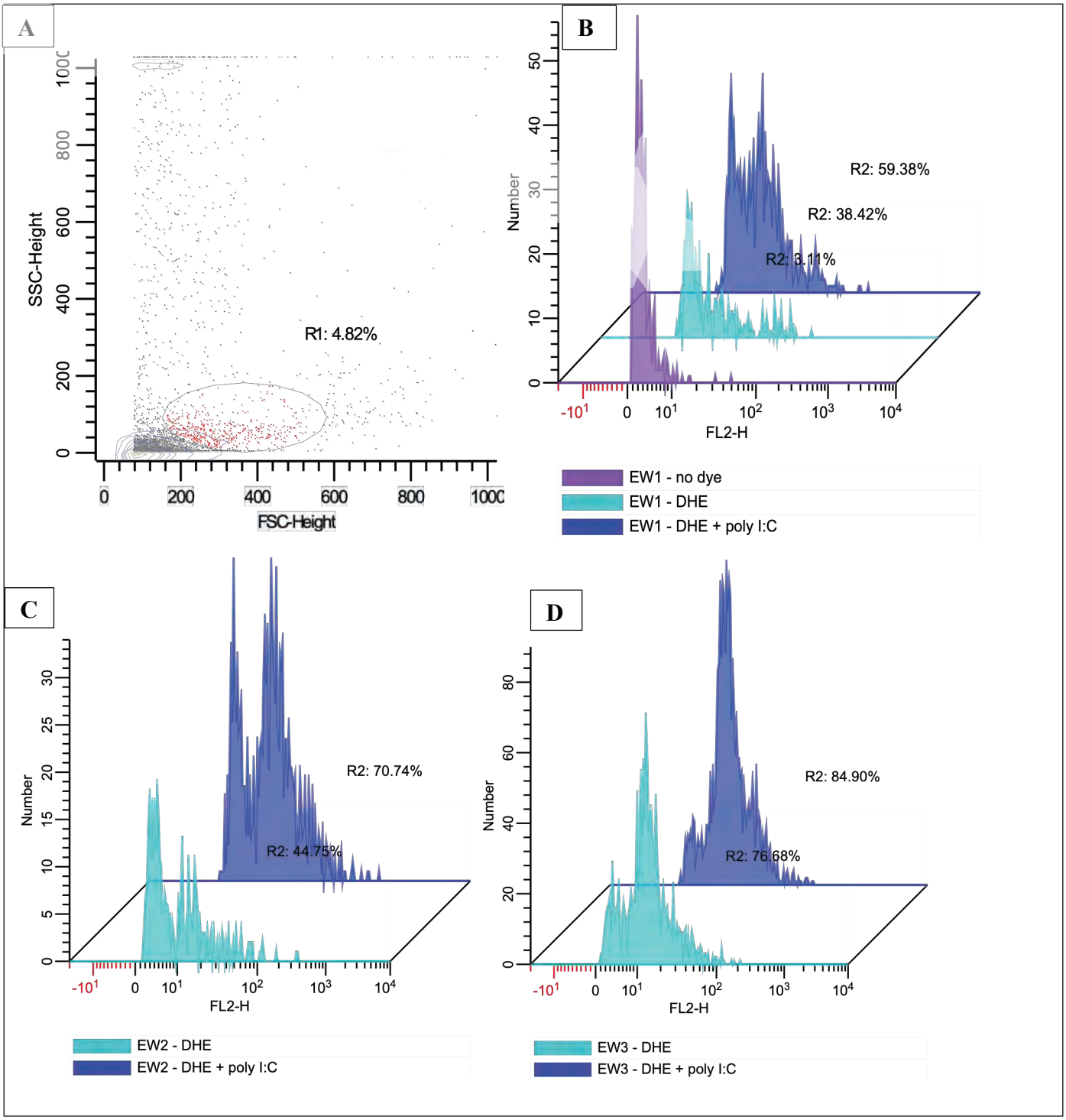

Figure 1. *WinList analysis of Assay a following flow cytometry.* **A.** FSC versus SSC graph shows the population of cells in region 1 (R1) selected for further analysis. The number of cells in R1 was between 200 and 600 for all samples. The percentage in R1 is equal to 4.82% for this representative sample. **B.** FL-2 overlay graph showing the percentages of coelomocytes in the no

dye (purple) control sample, the untreated (DHE-only) (turquoise) sample, and the poly (I:C) treated sample (poly (I:C) + DHE) (blue) for the three earthworm samples. The negative control exhibited 3.11% autofluorescence, the untreated sample had a 38.42% fluorescence (given that this sample was incubated with DHE, thus fluorescing more intensely than the negative no-dye control), while the treated one showed a 59.38% fluorescence. **C.** FL-2 overlay of the second earthworm sample. The untreated group exhibited a 44.75% fluorescence, while the treated illustrated a fluorescence of 70.74%. **D.** FL-2 overlay of the third earthworm sample. The untreated sample exhibited 76.68% of fluorescence, compared to the treated one that had a fluorescence of 84.90%.

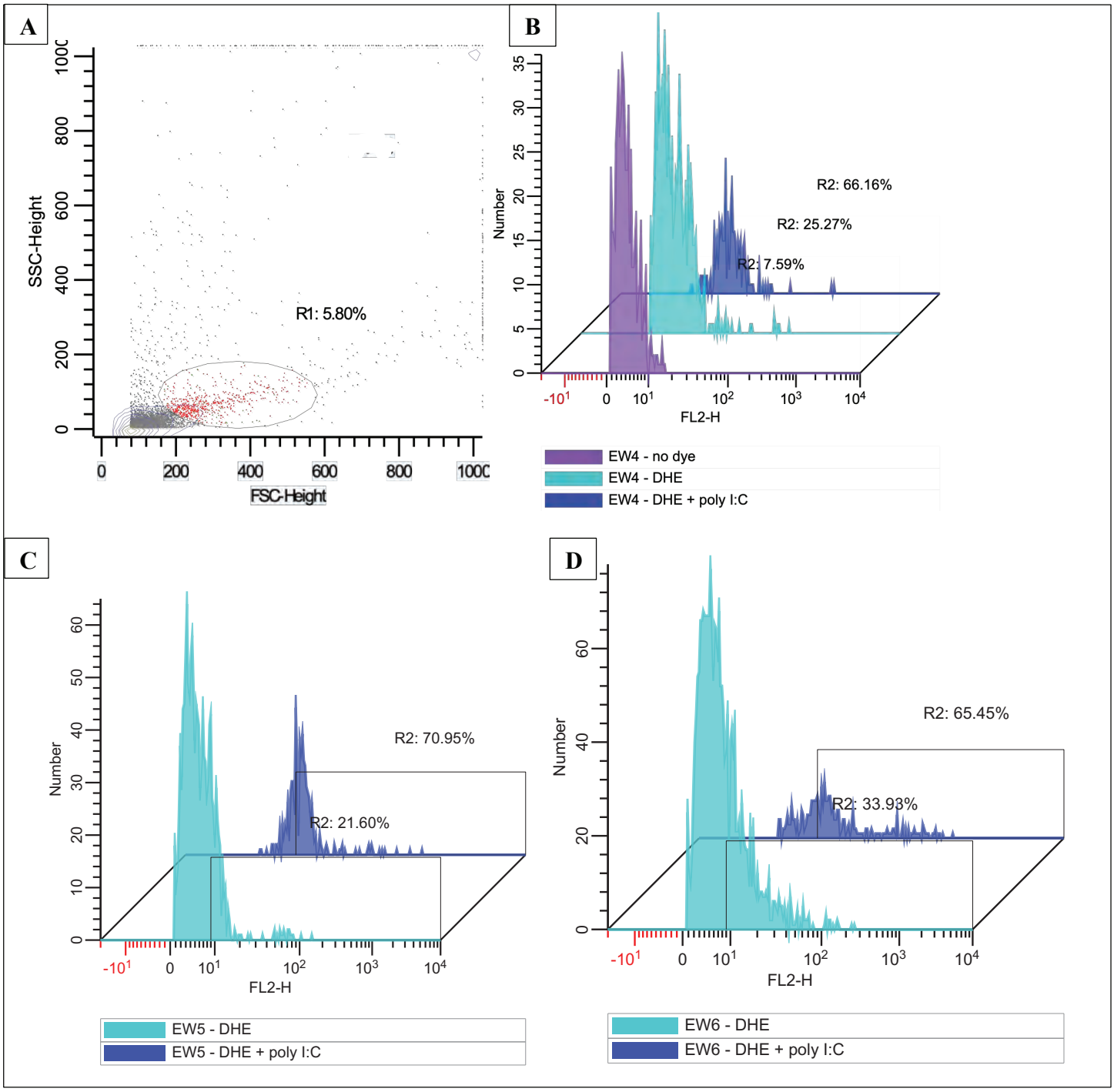

Figure 2. *WinList analysis of Assay b following flow cytometry.* **A.** FSC versus SSC graph depicting the population used for the second assay. The cells used for analysis are encircled in R1 holding between 200 and 600 hyaline amoebocytes. The percentage of cells further analyzed is equal to 5.80%. **B.** FL-2 overlay of the first earthworm sample. The negative control exhibited 7.59% autofluorescence. Fluorescence events for the untreated sample were 25.27%, while for the treated one were equal to 66.16%. **C.** FL-2 overlay of the second earthworm sample. The

untreated sample showed 21.60% of fluorescence events, compared to the treated sample that reached as high as 70.95%. **D.** FL-2 overlay of the third earthworm sample. The untreated sample demonstrated 33.93% of fluorescence events, while the treated sample reached to 65.45%.

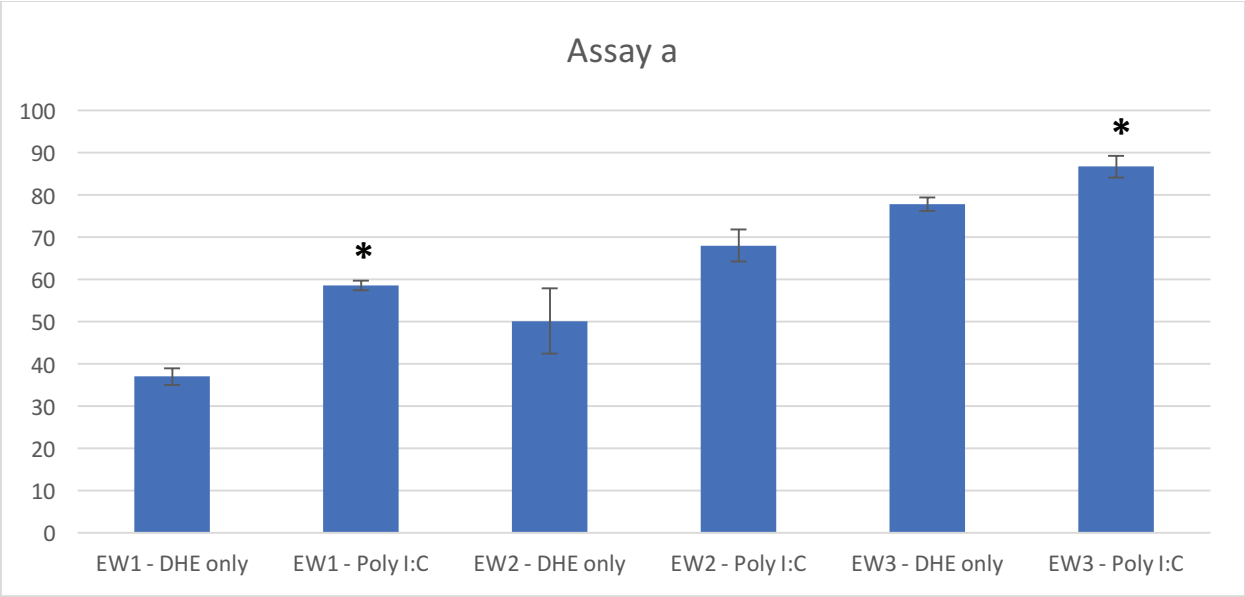

Figure 3. *Graphic representation of the significance of the results derived from Assay a.* This bar graph compares the results of the untreated (DHE only) sample with the treated sample (DHE + poly (I:C)). Following assay development in duplicates, flow cytometry, and WinList analysis, the findings were assessed for their significance by running a student's t-test paired two sample for means. The y axis indicates % FL-2 positive events in R2, while the x axis shows coelomocyte samples from three different earthworms (EW1-EW3), and their respective treatment. In all cases, when comparing the untreated sample to the treated one, there is some increase in the percentage of fluorescent cells in R2. However, only the results derived from EW1 and EW3 were significant (* = $p < 0.05$).

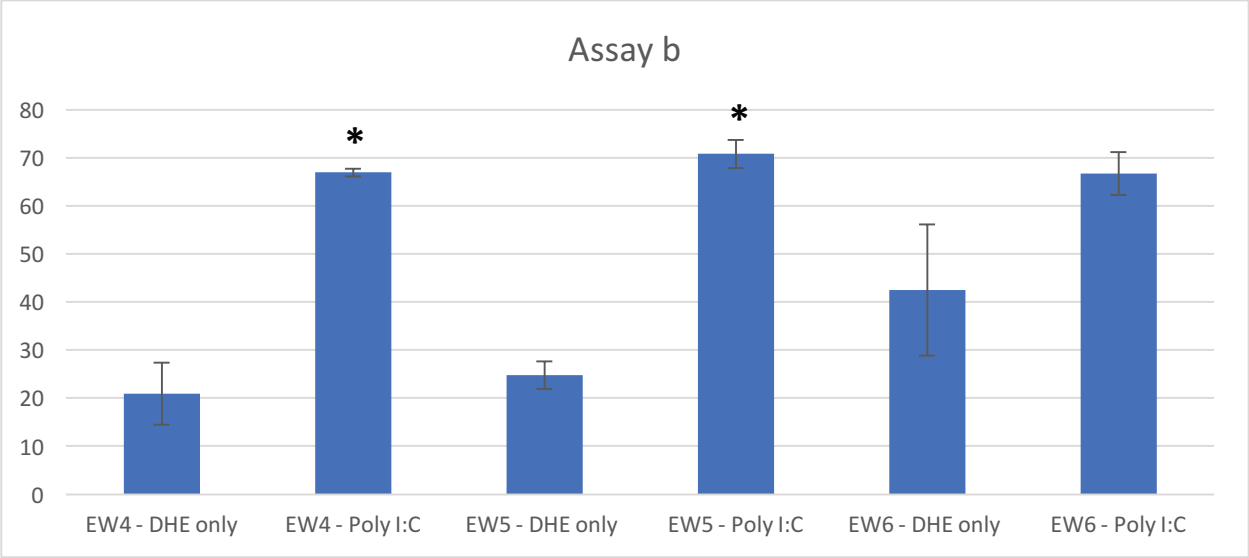

Figure 4. *Graphic representation of the significance of the results derived from Assay b.* This bar graph compares the results of the second assay, with the purpose of evaluating the significance of the findings. Following assay development in triplicates, flow cytometry, and WinList analysis, the findings were assessed for their significance by running a student's t-test paired two sample for means. The y axis indicates % FL-2 positive events in R2, while the x axis shows coelomocyte samples from three different earthworms (EW4-EW6), and their respective treatment. While there seems to be a great increase in the percentage of fluorescence events in the treated sample, only the findings for EW4 and EW5 are significant (* = $p < 0.05$).

Student Perceptions of Public Safety Compared to Municipal Police

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Abstract

Public safety departments on college campuses are an extremely under researched population. Though limited, previous research has explained that students value local law enforcement at a higher rate compared to public safety (Youstin & Kopp, 2021). In addition, students expected public safety to protect them but not interfere with their general college experience. In the spring semester of 2023, Cabrini University students were surveyed on their perceptions of these two law enforcement entities via an online Google Forms survey. The survey was distributed to students by their professors using a link or QR code. This study compares the perceptions of municipal police to public safety amongst college students. The sample size amounted to 59 respondents, (n=59). The results show that the hypothesis was not supported by the research which stated that views of public safety will influence the views of municipal police. However, a person's interactions with public safety was significant toward their perceptions of campus safety. Also, a student's race was statistically significant toward one's views of municipal police. In conclusion, public safety cannot be compared to municipal police in terms of perceptions.

Introduction

Law enforcement is a system that keeps peace and order within a society. Policing within the United States has had a long and difficult history due to fluctuations of support over the years (Parker & Hurst, 2021). Throughout history, numerous levels of law enforcement have emerged such as local, state, federal and university police which all serve their own purposes within their jurisdiction. Correspondingly, the duties for all law enforcement are to protect and serve, but it is the age group at which they serve that makes the distinction between local and public safety. Specifically, campus police are a specialized group of officers that only possess jurisdiction over a university's property also known as public safety. Overall, public safety is an extremely under researched population and more research should be done due to the popularity and relevance that safety holds on campuses (Aiello, 2020). College is supposed to be a safe place with little crime that is also separate from the local law enforcement's daily patrol. College students live and interact daily with public safety and as soon as they leave campus, they enter municipal police jurisdiction. Since municipal and public safety operate in the same geographical region,

communication and collaboration is constantly present which highlights the intersection these two departments face.

Moreover, the culture surrounding policing is attempting to shift toward a more progressive viewpoint. The current climate of society and the way law enforcement operates now is affecting general perceptions (Schoenle, 2017). General perceptions are the way that society as a whole view police and what their level of support is. There has been limited research conducted on the differences between municipal police and campus police, especially the perceptions that students possess. Though limited, previous research has explained that students value local law enforcement at a higher rate compared to public safety (Youstin & Kopp, 2021). Furthermore, support for law enforcement has varied throughout the years especially among young adults, 18 to 29 years old. This age group supported the lowest percentage of funding to police compared to those 50 years and older (Parker & Hurst, 2021). This poses an issue that young adults of this generation do not support the police which might escalate into poor perceptions of public safety. It is possible that perceptions of public safety may affect perceptions of municipal police. Comparing the differences between the two entities is essential to understanding how the public or students view these differences.

Literature Review

Police officers have been protecting citizens within the United States of America for decades using the Procedural Justice Theory Model. This theory became the framework in which a majority of modern-day policing derives from, it is broken into two areas. These cover the quality of decision making by officers as well as the quality of treatment of individuals (Aiello, 2020). The model was designed for municipal law enforcement to structure their daily duties,

into being community oriented, which became known as community policing. Also, this theory affects the way that people perceive police due to the officer's decision making and general treatment of the public. The various levels of law enforcement differ in jurisdiction and location; however, they also vary in public perceptions of police. As mentioned previously, the overall level of support generally varies by the age of the person perceiving them. A person's perception affects their general attitudes through differences in behaviors. The level of service provided by the law enforcement officers tends to be the highest determinant that affects one's attitudes toward officers (Youstin & Kopp, 2021).

Municipal Police

The services for municipal law enforcement can range from traffic stops to detectives conducting full investigations or making arrests. Municipal police's general duties are making arresting, enforcing traffic violations, conducting investigations as well as enforcing laws. Officers also work to prevent crimes and deterring criminal activity which can be seen as peacekeeping (Armstrong et al., 2021). All branches of law enforcement assist each other which naturally creates influence on one another (The United States Department of Justice, n.d.).

Municipal police (MP), also known as local police, is the most well-known form of policing due to the rate of interaction and public face this law enforcement level holds. Working in all areas within their jurisdiction allows officers to experience a large variety of crimes that may be more serious in nature (Allen, 2021). A comprehensive outline of serious crimes may entail the responsibilities to respond to large scale serious crimes such as massive events, terroristic attacks, devastating car crashes and higher levels of crime like murders and rape. With these larger crimes collaboration is often present with other levels of law enforcement such as

state or federal agencies (Armstrong et al., 2021). When necessary, jurisdictions overlap leading municipal police to lean on public safety for support.

Public Safety

Campus police made its first debut on Yale University's Campus in 1894 with only two full time officers (Patten et. al, 2016). From here, university police officers have become the norm across the United States to keep students safe while they obtain degrees (Allen, 2017). Campus safety officers have evolved into various forms such as public safety officers or formal university police officers, in order to keep up with the ever-changing development of college society. As of now the University of Pennsylvania has the largest university police force with 121 sworn officers (Division of Public Safety, 2022). Students expect public safety to protect them but not interfere with their general college experience (Youstin & Kopp, 2021); meaning that public safety has a responsibility to keep the students safe. On the contrary, students naturally desire the freedom associated with college and being an adult. In addition, one of the largest determining factors of attitudes toward law enforcement was the service provided by the officers (Youstin & Kopp, 2021).

In addition, public safety (PS) is a branch of authority that has jurisdiction on college and university campuses. The need for public safety rose exponentially after the first mass shooting on a college campus in the 1960, known as the University of Texas Tower shooting. During the 1980s, the necessity for university police rose again after the death of Jeanne Ann Clery who was murdered on campus (Allen, 2021). After these tragic events, public safety shifted to follow a community policing style in which the department would take a proactive approach opposed to a reactive one. As of today, the role of public safety is to ensure the safety of students on campus

by responding to calls, enforcing parking violations and providing security for events (Cabrini University Department of Public Safety, 2022).

Moreover, students claimed that public safety's job is to enforce and discipline the activities that college students find fun such as drinking and partying (Allen, 2017). College is a time to branch out and have new experiences, but with this newfound freedom comes the expectation of safety. It is the duty of public safety to provide this safety to students. PS must balance the wishes of administration, studies, and board members, making their job a tough balancing act (Allen, 2021). Students have reported that the perceptions of PS are as follows; a study found that 31% of students stated that university police "ruin their fun" while the majority at 69% showed that they do not ruin the fun (Allen, 2017). The way the students perceive the public safety officer's job influences one's support toward them.

Public Perceptions of Police

The public view of law enforcement plays a pivotal role in each department through funding and general cooperation of the community (Youstin & Kopp, 2021). The various levels of policing such as state or local police are rooted in reactions to the public's call for help. If the public is not in support of police, then they will be less likely to call for assistance when needed, in accordance with the Procedural Justice Model (Aiello, 2020). An increased perception will increase an individual's desire to call law enforcement when they are in need. Citizens that perceive the police as helpful are at an increase to willingly engage or cooperate with authorities (Tyler, 2009). This concept above outlines that trust is the basis for a strong police-community relationship.

To specifically understand the perceptions of MP, a study that looked at the demographic makeup of my person compared those demographics toward support. The researchers wanted to

see which trait affected their support the most out of age, race, marital status, and gender. The results of the study showed that African American, males and single students reported negative views on police officers (Armstrong et al., 2021). Many social issues, such as “Black Lives Matter" and many other civil movements on the topic of police brutality affect one’s perception of police effectiveness. A researcher by the name of Tom Tyler explained that perceptions of police rely on a person’s preconceived notions of police tendencies and some characteristics are race and age. Our current societal climate is one with a decreasing view of police effectiveness (Columbia Southern University, 2022). The media plays a driving role within these perceptions.

Perceptions of police often stem from the media and how individuals consume the media. McLuhan’s theory of communication states that the media has social consequences, are extensions of one’s senses, and that the media can manipulate perceptions of space and time (Miani, n.d.). It can be seen through this theory that the media is the message which affects one’s perceptions or understandings of a topic. The way a person consumes their media and the platform in which they view it can have a lasting impact on their perception. Recently, policing has been a hot topic within the news and people are consuming the headline stories about police officers. This is affecting their perceptions and reactions to officers all over the world.

Perceptions of Public Safety amongst College Students

Attending college is a pivotal time in a person’s life and choosing a college is a great responsibility. Students choose to attend a certain college for numerous reasons such as major/program, location, and financial aid. However, when a student is deciding upon a specific university that fits their needs, often safety is a factor (Puckett, 2022). Universities are required to publicly post their crime statistics due to the Clery Act. The Jeanne Clery Disclosure of Campus Security Policy and Campus Crime Statistics was implemented after the murder of

Jeanne Clery on Lehigh University's campus. The parents of the late student claimed that if they knew the crime rates within the area that they would not have sent their daughter to that university (LSU Health Shreveport, n.d.). However, even with the implementation of the Clery Act, less attention has been focused on how security of the campus plays a role in college choice (Puckett, 2022).

Conversely, the results of a study conducted at East Tennessee University explained that students are less likely "to care about the availability of information pertaining to crime and safety on college/university websites." (Puckett, 2022). This study focused on evaluating students' perceptions of safety and security on campus as well as the overall impact of perceptions of safety on enrollment. Parents view safety on campus as an essential deciding factor for their children (Puckett, 2022). Amongst the student body itself, it was found that students who are older are at a greater likelihood to report increased levels of satisfaction when compared to their younger counterparts. Due to this, age is a factor amongst perceptions of campus safety. Public safety should be a resource that all ages of students should be satisfied with and building trust is important to a safe university community. From the previous literature it is reported that public safety could improve on several topics, such as providing students with crime and safety information. Similarly, the students showed decreased confidence in an officers' ability to investigate any sort of reported crime.

Oftentimes, the lines of police blur when partnerships are created. An example is when a public safety officer called the local police for assistance on an investigation or arrest. This is when jurisdictions begin to blur in the public eye which causes any feeling toward municipal police to spill over into perception of public safety. Students perceive police officers as ruining their fun and "bullies, with nothing better to do" (Allen, 2017). As mentioned previously,

perception varies by age and 18–29-year-olds have the least amount of respect for officers (Parker & Hurst, 2021). There is little known about public safety offices in terms of the perceptions of them amongst college students. This study is meant to identify the presence or lack of connection between student's perceptions of municipal police officers compared to public safety officers. I expect to find a relation between these two law enforcement entities.

Hypothesis:

Perceptions of public safety whether positive or negative will influence perceptions of municipal police.

- If an individual has a negative outlook on public safety officers, then they will look less favorably on municipal officers as well.
- If a student has a positive view on public safety officers, then they will view municipal officers as positive.

Methodology

Procedure

The principal researcher surveyed the Cabrini University undergraduate student population to conceptualize the factors that predict perceptions of public safety compared to municipal law enforcement. This survey took place on Cabrini University's campus in Radnor, Pennsylvania in the spring semester of 2023. Cabrini University is a small Catholic institution with Division III athletics in a suburban area. The data for the study was conducted by using the availability sampling method. The study was conducted with the use of online survey questionnaires through the application Google Forms. The research topic and survey questions were approved by the Cabrini University Institutional Review Board prior to the data collection.

The survey was distributed to students by numerous avenues. First, professors within several departments such the Department of Sociology, Criminology, and Justice as well as the Psychology Department were contacted by the researchers to receive permission to use available class time. Next, the researchers physically went into classrooms and explained the purpose of the study to have students participate then provide the link to the online survey. Lastly, the survey was sent to associates of the researchers via text messages that contained the online survey link.

Before participants could complete the survey, the respondents were required to sign a consent form. The form included the purpose of the research, the participants' rights, and the primary researchers' contact information. The respondents who received a link were informed that they survey was voluntary and offered no incentive toward them. Since the survey had multiple topics within it the participants were altered of the sensitive nature of the research within the consent form. If a participant felt bothered by the survey, they were provided with contact information of the psychological services on Cabrini's campus. Participants were reminded that data was both confidential and anonymous. All raw data from the survey was deleted off of the researcher's computer once analysis was complete.

Sample Demographics

The survey yielded a sample size of 59 students from Cabrini University. Demographic questions were asked of students such as gender, age, class year, race, and ethnicity. After running SPSS frequency distributions for the variable of "Gender" the gender split of respondents is something to note. The sample consisted of 76.3% (n= 45) cisgender females, 18.6% (n= 11) cisgender males, and 5.1% (n= 3) Non-Binary, Third Gender, or Gender Queer individuals. The mean was female, showing a majority of respondents identified as cisgender

female. Age was the next question asked on the survey. The sample consisted of 10.2% (n= 6) 18-year old's, 18.6% (n= 11) 19-year old's, 23.7% (n= 14) 20-year old's, 33.9% (n= 20) 21-year old's, 11.9% (n= 7) 22-year old's and 1.7% (n= 1) 23-year-old. The mean was 21 year olds. The class year of the respondents was asked as well. The sample consisted of 18.6% (n= 11) freshman, 18.6% (n= 11) sophomores, 37.3% (n= 22) juniors, and 23.7% (n= 14) seniors. The most common response was juniors.

In addition, respondents were able to self-identify their race. The sample consisted of 69.5% (n= 41) White, 10.2% (n= 6) Black or African Americans, 5.1% (n= 3) American Indian/Alaska Native, Native Hawaiian, or other Pacific Islander, 13.6% (n= 8) Asians, 1.7% (n= 1) another race not specially listed previously/ other. The mean was white, meaning a majority of the respondents self-identified as white. Ethnicity was the final demographic question on the survey. The sample consisted of 20.7% (n= 12) Hispanic Latino/, and 79.3% (n= 46) Non-Hispanic or Latino/a. Table 1 compiles all the demographic data listed above. The mean was non-Hispanic or Latina students.

Table 1
Sample Demographics

	Frequency	*Valid Percent*
Gender		
Female	45	76.3
Male	11	18.6
Other Gender	3	5.1
Age		
18	6	10.2
19	11	18.6
20	14	23.7
21	20	33.9
22	7	11.9
23	1	1.7
Class year		
Freshman	11	18.6
Sophomore	11	18.6

Junior	22	37.3
Senior	14	23.7
Race		
White	41	69.5
Black or African American	6	10.2
Other Pacific Islander	3	5.1
Asian	8	13.6
Other	1	1.7
Ethnicity		
Hispanic or Latino/a	12	20.7
Non-Hispanic or Latino/a	46	79.3

Other Gender= Non-Binary/Third/ Gender/Gender/Queer
Other Pacific Islander= American Indian/Alaskan Native/ Native Hawaiian or

Independent Variable: Public Safety

Perceptions public safety was the independent variable for this study. Public safety was defined as a service agency that functions as part of the university. Security personnel do not have full police powers and rely on municipal, county, or state police for support in criminal matters." (Department of Public Safety, n.d.). This definition was listed on the survey within the instructions section so students could reference it throughout the survey completion.

Respondents were asked to answer a series of the following five questions that related to the independent variable under the survey section titled “Perception of Campus Public Safety and Municipal Police”. An example of a question is as follows: Would you agree with the statement that “public safety is a helpful resource on campus”? The responses to the question are as follows: 1= strongly agree 10.2% (n= 6), 2= agree 23.7% (n= 14), 3= neither agree nor disagree 18.6% (n= 11), 4= disagree 42.4% (n= 25), 5= strongly disagree 5.1% (n= 3). A majority of students remained neural to this question showing that they do not have strong opinions regarding the helpfulness of public safety, (M= 3.08, SD= 1.13).

Table 2
Frequencies for the Independent Variable of Public Safety

	Frequency	***Valid Percent***
Helpfulness of Public Safety		
Strongly Agree	6	10.2
Agree	14	23.7
Neither agree nor disagree	11	18.6
Disagree	25	42.2
Strongly Disagree	3	51.1
Contact with Public Safety		
0 times	6	10.2
1-2 times	11	18.6
3-4 times	14	23.7
5-6 times	20	33.9
7 or more times	7	11.9
Interaction Satisfaction		
Very satisfied	0	0.0
Satisfied	20	33.9
Neutral	13	22.0
Dissatisfied	8	13.6
Very dissatisfied	0	0.0
Views of Public Safety		
Mostly positive	8	13.6
Positive	22	37.3
Neutral	19	32.2
Negative	5	8.5
Most negative	0	0.0
Equally Important Departments		
Yes	17	28.8
No	28	47.5
Neutral	14	23.7
Influence		
Yes	4	6.8
No	40	67.8
Neutral	15	25.4

Dependent Variable: Municipal Police

Perceptions municipal police was the dependent variable within the study. The municipal police are conceptualized as follows: “any natural person who is properly employed by a municipality, including a home rule municipality, as a regular full-time or part-time police

officer" (Pennsylvania State Police & MPOETC, n.d.). For the purpose of the survey, this is the way that municipal police were defined, and the definition was listed under the survey for reference.

Respondents were asked to answer a series of the following four questions that related to the dependent variable. An example of a question is as follows: How many times, in the past 12 months have you had contact with a municipal police officer for a traffic violation, emergency situation, non-emergency contact or other interactions (e.g., attend a community meeting or talk to an officer on patrol)? The responses to the question are as follows: 1= 0 times 55.9% (n=33), 2= 1-2 times 37.3% (n= 22), 3= 3-4 times 1.7% (n= 1), 4= 5-6 times 3.4% (n= 2), and 5= 7 or more times 1.7% (n= 1). Students interacted with municipal police approximately 1-2 times on average, (M= 1.58, SD= .835).

Table 3

Frequencies for the Dependent Variable of Municipal Police

	Frequency	***Valid Percent***
Contact with Municipal Police		
0 times	33	55.9
1-2 times	22	37.3
3-4 times	1	1.7
5-6 times	2	3.4
7 or more times	1	1.7
Interaction Satisfaction		
Very satisfied	3	7.7
Satisfied	12	30.8
Neutral	19	48.7
Dissatisfied	4	10.3
Very dissatisfied	1	2.6
Views of Public Safety		
Mostly positive	7	11.9
Positive	17	28.8
Neutral	25	42.4
Negative	5	8.5
Most negative	4	6.8

Plans for Analysis

As stated within the literature review, the hypothesis for this study is that the perceptions of public safety whether positive or negative will influence perceptions of municipal police. If an individual has a negative outlook on public safety officers, then they will look less favorably on municipal officers as well. If a student has a positive view on public safety officers, then they will view municipal officers as positive. In order to test this hypothesis with the data that was gathered, three statistical tests will be run which include a Chi Squared, a Spearman's Correlation and Pearson's Correlation.

By definition, a Chi Squared analysis tests the hypotheses by checking the observed frequencies and match them to the expected frequencies. This test requires the independent variable to be nominal or ordinal. A Spearman Correlation analysis uses rank/ order data in order to test the strength and direction of the two variables. The variables must be interval or ratio data. A Pearson's Correlation is similar to a Spearman's correlation in the sense that it test the strength and direction of the two variables (Bevans, 2022). These variables must also be interval or ratio.

Results

For reference, the research question was "Do student's views of Public Safety influence their views of Municipal Police?". The hypothesis stated that the perceptions of one will affect the perceptions of the other. However, the data gathered did not support the hypothesis meaning that views of municipal police were not significantly correlated with one's views of public safety. To test this research question, a Spearman Correlation analysis was run using the data gathered from the researcher's survey. The independent variables within this test was views of public

safety while the dependent variable was views of municipal police. Both the independent and dependent variable were interval data making a Spearman Correlation appropriate in this case. According to Table 4, there was a weak negative correlation found; r(58)=.129, p>.05. The way in which one views public safety does not statically influence their views of municipal police. However, Table 4 did find that one's satisfaction with their public safety interactions affects their views of campus public safety; r(58)= .479, p<.05. This was a moderate positive correlation.

Table 4
Views of Public Safety versus Municipal Police (N=58)

Variables	***(1)***	***(2)***	***(3)***
1) Views of Municipal Police	1	-	-
2) Satisfaction with Public Safety Interactions	-.043	1	-
3)Views of Campus Safety	.129	.479**	1

**p<.05

A Spearman correlation was run in order to test the four questions associated with the two variables of public safety and municipal police. This correlation is meant to strength and direction of the two variables similar to the Pearson's Correlation. The independent variables within this test was views of public safety while the dependent variable was views of municipal police. As reported by Table 5, a person's level of satisfaction with their interactions with the public safety department is significantly correlated to one's overall view of public safety at a moderate positive correlation, which was previously identified within Table 5, r(58)= .525, p<.05. All other variables were not significant to each other.

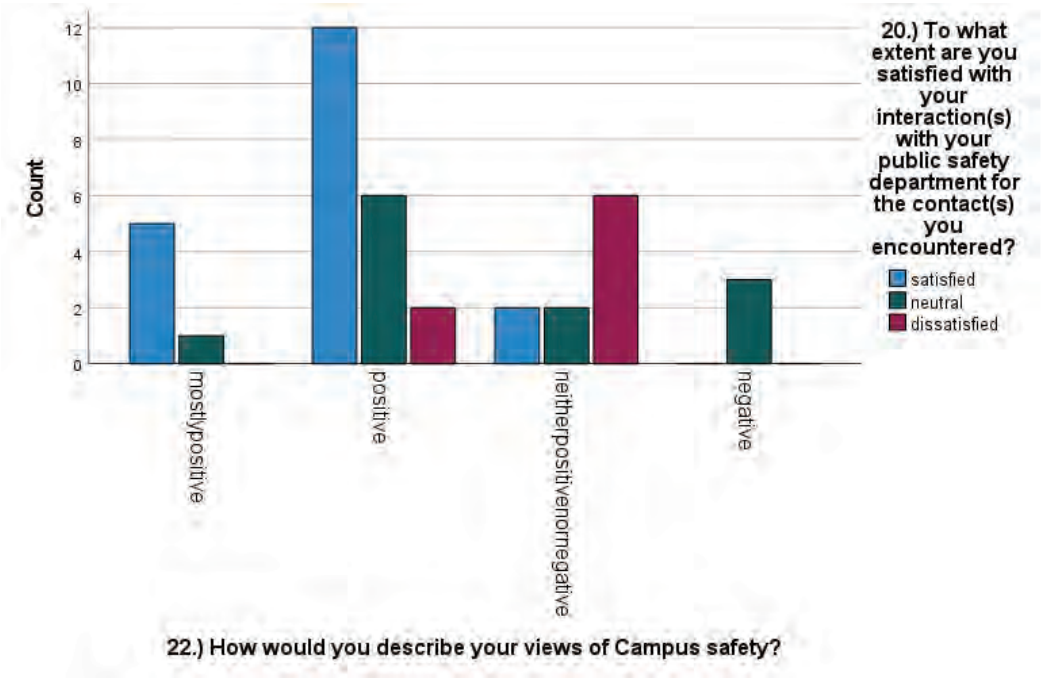
Table 5
Views and Satisfaction between Public Safety and Municipal Police (N=58)

Variables	***(1)***	***(2)***	***(3)***	***(4)***
1) Views of Municipal Police	1	-	-	
2) Views of Campus Safety	.008	1	-	
3)Satisfaction with Public Safety Interactions	.106	.525**	1	
4) Satisfaction with Municipal Police Interactions	.099	-.157	-.027	1

**p<.05

Using the independent variables of views of public safety and the dependent variable of views of municipal police, Figure 1 was created to represent student's views of public safety. The data was gathered from the survey called "College Students and Perception on Police, Stress, and Rape Myths" that was used to craft this bar graph. Views of campus safety run along the x axis and one's satisfaction with their public safety interactions are along the y axis. Shown in the color blue, a large portion of students are satisfied with their interactions forming a positive view of public safety officers. Across the entire graph, several students felt neutral toward both views of campus safety and their personal interactions.

Figure 1
Views of Campus Safety



Gender was compared to views of municipal police on a scale from mostly positive to mostly negative. According to Table 6, gender did not seem to play a huge role amongst one's views of police. However, there was an overwhelming number of respondents who identified as female. 43% of females and 36% of males choose the neutral option to this question of their views of police. Lastly, those who identified as non-binary, third gender or gender queer seemed to look less favorably on municipal police or remained neutral.

Table 6
Frequency of Views of Municipal Police and Gender (N=58)

Gender	***Mostly Positive***	***Positive***	***Neither Positive nor Negative***	***Negative***	***Mostly Negative***	***Total***
Female	11.4% (5)	29.5% (1)	43.2% (19)	11.4% (5)	4.5% (2)	(44)
Male	18.2% (2)	36.4% (4)	36.4% (4)	0.0% (0)	9.1% (1)	(11)
Other	0.0% (0)	0.0% (0)	66.7% (2)	0.0% (0)	33.1% (1)	(3)
Total	12.1% (7)	29.3% (17)	43.1% (25)	8.6% (5)	6.9% (4)	58

**"Other" =Non-Binary/ Third Gender/ Gender Queer

In order to statistically identify if gender impacted one's views of municipal police a Chi Squared test was run. A Chi Squared test was appropriate in order to compare the variables of "gender" and "views of police" which compared demographics to the dependent variable of municipal police. Both of which are ordinal variables. A chi-squared test was run in order to attempt to find significance between gender and views of municipal police. There was no significance found between these two variables, x^2 (8, n=58) =7.35, p=.499.

Table 7
Gender and Views of Municipal Police (N=59)

	Value	***df***	***Asymptotic Significance***
Pearson Chi-Squared	7.351	8	.499
Likelihood Ratio	8.221	8	.412
Linear-by-Linear Association	.562	1	.453
N of Valid Cases	58		

p<.05, Cramer's V Coefficient= .252, Pearson Chi Square= 7.35

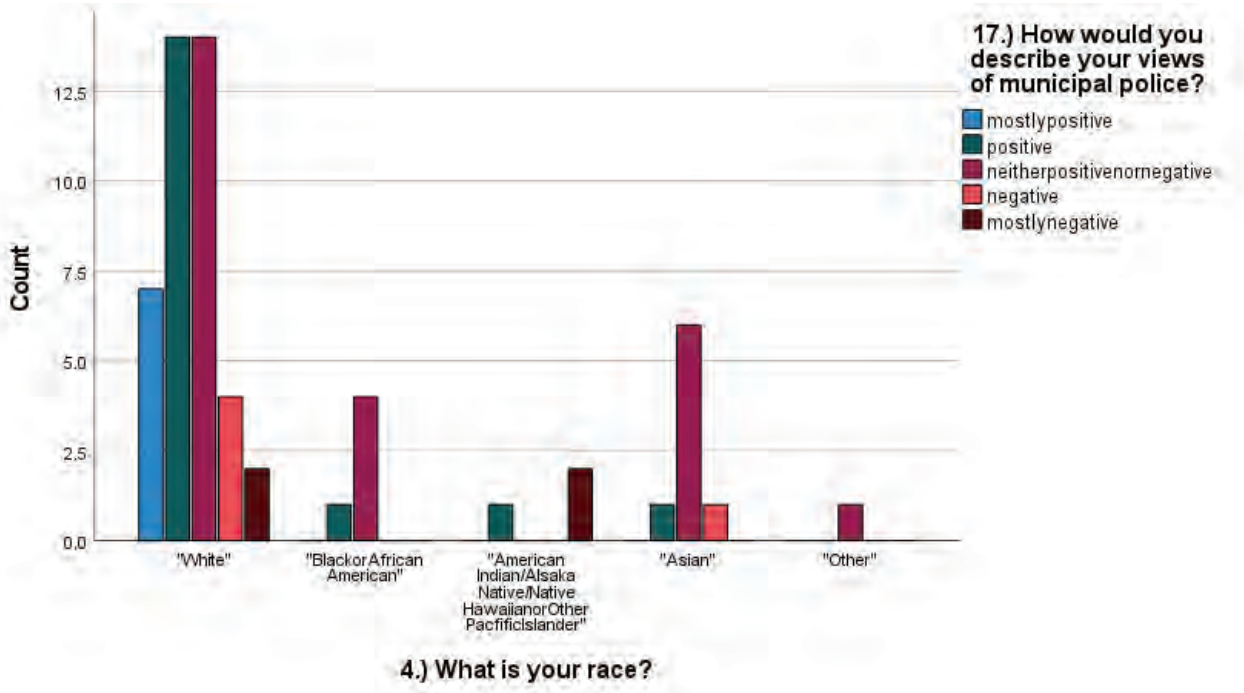
Race and views of police were compared to one another. Table 8 is a frequency table to show the views of municipal police compared to one's race. The findings below shows that out of the five Black or African American student respondents, 80% of then answered their views as neither positive nor negative, or neutral. 28 respondents who self-identified as "white" selected positive or neutral when asked about their views of Police. Six out of the eight respondents who self-identified as Asian, selected neutral for their views.

Table 8
Frequency of Race and Views of Police

Race	***Mostly Positive***	***Positive***	***Neither Positive nor Negative***	***Negative***	***Mostly Negative***	***Total***
White	17.1% (7)	34.1% (14)	34.1% (14)	9.8% (4)	4.9% (2)	41
Black or African American	0.0% (0)	20.0% (1)	80.0% (4)	0.0% (0)	0.0% (0)	5
American Indian/ Alaskan Native/Native Hawaiian or Other Pacific Islander	0.0% (0)	33.0% (1)	0.0% (0)	0.0% (0)	66.7% (2)	3
Asian	0.0% (0)	12.5% (1)	75.0% (6)	12.5% (1)	0.0% (0)	8
Other	0.0% (0)	0.0% (0)	100.0% (1)	0.0% (0)	0.0% (0)	1
Total	7	17	25	5	4	58

Figure 2 is a bar graph that is a visual representation of the race compared to the views that students possess regarding municipal police. The data used to form this graph came from the state used in Table 8. Since the majority of the respondents to this survey self-identified as white those columns are at the providing the bulk of the data. All other information regarding the statistical information of race and views of police can be found under Table 8.

Figure 2
Race and Views of Municipal Police



To compare the relationship between the demographic variable of race and the dependent variable of views of municipal police, a Chi-Squared test was used. In accord to the Chi-Squared test run below in Table 9, it demonstrates that a person's race is statically significant toward one's views on municipal police; x^2 (16, n=58) =28.46, p=.028. This result will be discussed further under the conclusion.

Table 9
Race and Views of Municipal Police

	Value	***df***	***Asymptotic Significance***
Pearson Chi-Square	28.458	16	.028
Likelihood Ratio	23.098	16	.111
Linear-by-Linear Association	3.504	1	.061
N of Valid Cases	58		

p<.05, Cramer's V Coefficient= .350

Table 10 is a frequency distribution that shows the age of the respondent compared to the person' views of municipal police. The majority of respondents (n= 25) regardless of age, self-reported that they felt neutral to this question. In short, one's age does not appear to play a role in their views of police.

Table 10
Frequency of Age and views of police

Age	*Mostly Positive*	*Positive*	*Neither Positive nor Negative*	*Negative*	*Mostly Negative*	*Total*
18-20	10.0% (3)	36.7% (11)	36.7% (11)	10.0% (3)	6.7% (2)	30
21-23	14.3% (4)	21.4% (6)	50.0% (14)	7.1% (2)	7.1% (2)	28
Total	12.1% (7)	29.3% (17)	43.1% (25)	8.6% (5)	6.9% (4)	58

A chi squared test was ran to potentially identify a relationship between the variables of "age" and "views of police". This relationship was found to not be significant; x^2 (20, n=58) =20.848, p=.406, meaning that age does not relate to one's views of police.

Table 11
Age and Views of Municipal Police

	Value	*df*	*Asymptotic Significance*
Pearson Chi-Square	20.848	20	.406
Likelihood Ratio	22.818	20	.298
Linear-by-Linear Association	.005	1	.946
N of Valid Cases	58		

Cramer's V=.300 and sig .406

Table 12 is a frequency distribution that shows the "class year" of the respondent compared to the person's "views of municipal police". The majority of respondents (n= 25)

regardless of class year, self-reported that they felt neutral to this question. One's class year does not appear to play a role in their views of police.

Table 12
Frequency of Class Year and Views of Municipal Police

Class Year	***Mostly Positive***	***Positive***	***Neither Positive nor Negative***	***Negative***	***Mostly Negative***	***Total***
Freshman	0.0% (0)	18.2% (2)	63.6% (7)	9.1% (1)	9.1% (1)	11
Sophomore	9.1% (1)	54.5% (6)	18.2% (2)	9.1% (1)	9.1% (1)	11
Junior	19.0% (4)	28.6% (6)	38.1% (8)	9.5% (2)	4.8% (1)	21
Senior	14.3% (2)	21.4% (3)	57.1% (8)	0.0% (0)	7.1% (1)	14
Total	12.3% (7)	29.8% (17)	43.9% (25)	7.0% (4)	7.0% (4)	58

In order to potentially identify a relationship between the variables of "class year" and "views of police", a chi squared test was ran. This relationship was found to not be significant; x^2 (12, n=57) =10.316, p=.588. According to Table 13, there was no significance shown between one's views of police and grade in school.

Table 13
Class Year and Views of Municipal Police

	Value	***df***	***Asymptotic Significance***
Pearson Chi-Square	10.316	12	.588
Likelihood Ratio	12.500	12	.406
Linear-by-Linear Association	1.058	1	.304
N of Valid Cases	57		

Crammers V= .246 and significance= .588

Table 14 is a frequency distribution that shows one's "ethnicity" compared to the person's "views of municipal police". The majority of respondents (n= 25) regardless of class

year, self-reported that they felt neutral to this question. One's ethnicity either Hispanic or non-Hispanic, does not appear to play a role in their views of police.

Table 14
Ethnicity and Views of Municipal Police

Ethnicity	***Mostly Positive***	***Positive***	***Neither Positive nor Negative***	***Negative***	***Mostly Negative***	***Total***
Hispanic or Latino/a	8.3% (1)	16.7% (2)	66.7% (8)	8.3% (1)	0.0% (0)	12
Non-Hispanic or Latino/a	13.3% (6)	31.1% (14)	37.8% (27)	8.9% (4)	8.9% (4)	45
Total	12.3% (7)	28.1% (16)	43.9% (25)	8.8% (5)	7.0% (4)	57

A chi squared test was ran to identify a relationship between the variables of "ethnicity" and "views of police". This relationship was found to not be significant; x^2 (4, n=57) =3.770, p=.438. As shown in Table 15 listed below, there was no significance shown between one's views of police and ethnicity.

Table 15
Ethnicity and Views of Police

	Value	***df***	***Asymptotic Significance***
Pearson Chi-Square	3.770	4	.438
Likelihood Ratio	4.525	4	.340
Linear-by-Linear Association	.033	1	.856
N of Valid Cases	57		

Cramer's V= value .257 and significance .438

Discussion

Overall, the hypothesis of the study was not supported by the research. One's view of campus public safety does not seem to influence views of municipal police. This is most likely

due to the entities being vastly different. Public safety does not carry firearms or hold law-enforcement powers on campus while municipal police would. For example, using a sworn campus police department such as University of Pennsylvania police department to compare it to Philadelphia Police Department may provide different results. Making this comparison would be something of interest for future research.

As shown in Table 4, a student's views of public safety were significantly correlated with their interactions with public safety. Since Cabrini University public safety is not a large entity nor received much media attention, it makes sense to have one's personal experiences as a driving factor for their perceptions. This information would be beneficial for the Department of Public Safety to know that their perception stems from student's personal interactions with officers. Using this knowledge within officer training explaining how essential their interactions with students are could have a greater effect on their department.

In addition, race seemed to be the variable that effected perceptions of municipal police rather than opinions of public safety. A person's race is statically significant toward one's views on municipal police as found in Table 9. This result are backed by a study that was cited within the literature claiming that black and African American students have the least positive views of municipal police (Armstrong et al., 2021). The survey only showed 6 students who self-identified as black or African American. Cabrini University has a student population that consists of 48% BIPOC population (Cabrini University, n.d.). This was not represented by the data within this survey due to the fact that 69.5% of survey respondents identified as "white". In general, these results are pivotal in explain the racial disparities found within our society. These findings add to a lager body of race and police related research that could enhance the field. Seeing that a small campus like Cabrini can produce the same results as national studies is fascinating.

During edits, I re-ran the statistical test of a Spearman correlation to take into account the ordinal variable. Previously the responses for "satisfaction with public safety" was a nominal variable due to response of "I do not interact with public safety". After removing 20 responses of "I do not interact with public safety", the spearman correlation still produced the same significant results. In addition, the response "I do not interact with municipal police" was removed from "satisfaction with municipal police". However, there were zero responses for that option, allowing the test to be re-run out any numerical changes.

Limitations

There are several limitations to this study. The first being the low sample size, N=59 respondents. This sample size did not allow for a broad variety of students to respond, making the results interesting yet very difficult to generalize. In addition, all respondents were taken from Cabrini University which is a small Catholic school in Radnor, PA. The survey distributed to students contained three different question topics at contributed to three different students' research. The name of the overall survey was "Students' Perceptions of Police, Rape Myths and Safe Sex Practices". One of the reasons that the survey might not have gained a large number of responses is due to the fact there were sensitive questions about sexual education and rape myths. In accordance with a study conducted by The National Library of Medicine uncovered that those who completed the surveys on traumatic events reported more sadness if those participants had previously experienced a traumatic event (Labott, Et, al, 2013). With this in mind, I believe this was a true limitation that affected the number of responses I received for my specific survey questions relating to police perceptions.

Another potential limitation of the study was the discrepancy of responses across gender within the study. Shown in Table 7, a student's gender was not significantly correlated to one's

views of municipal police. Across both genders, a majority of responses, 25 out of 58, fell into the “neutral’ category of views, as shown in Table 6. Out of the total number of responses (N=59), 45 of those self-identified as cisgender female while only 11 respondents self-identified as cisgender male. In accord with the Cabrini University website, the average gender split is 65% women and 35% men (Cabrini University, n.d.). This statistic alone explains the gender split, and shows that any survey sent to Cabrini will likely receive more female responses. Also, the survey did not get sent out to the entire student body and there was no way to guarantee that all students see or take this survey. This disproportionate gender breakdown at Cabrini mostly likely contributed to the high number of women respondents. Since policing within our society is now a sensitive topic, it is possible that students felt indifferent or comfortable stating an opinion on this question.

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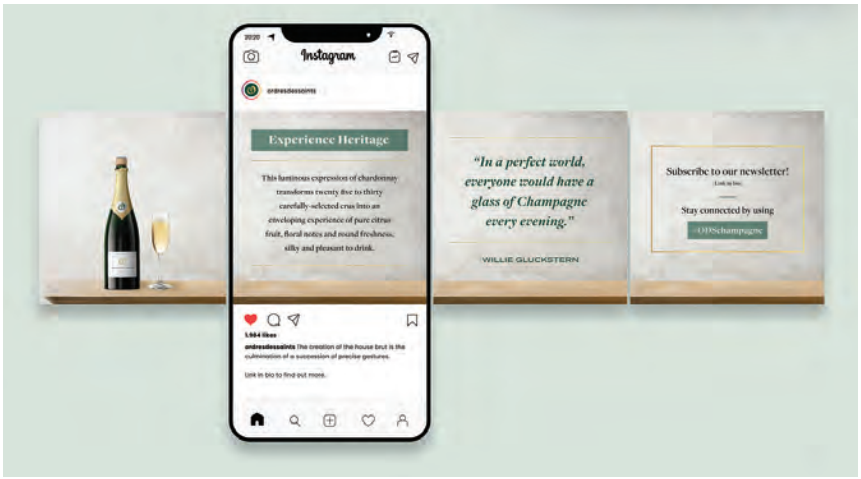

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