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Enumeration of Microbial Load in Used Mask

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ABSTRACT

Masks provide a simple barrier to prevent respiratory droplets spreading in the air. Respiratory infections can be transmitted through droplets of different sizes: when the droplets particles are >5-10µm in diameter referred to as respiratory droplets, and when the droplet particles are <5µm in diameter called as droplet nuclei. Masks can also be made of polystyrene, polycarbonate, polyethylene, polyester, cotton, wool, silk, linen or rayon. It creates humidity, thereby stops droplet from turning into droplet nuclei. The study was based on the survey carried out in two domains, i) mask wearing profile, ii) and mask wearing related knowledge and behavior. From survey respondents random samples of different mask were collected for enumeration of microorganisms. Survey questionnaire revealed that 86% of participants prefer reusage of masks, 16% participants used the same mask more than 6hours in a day, out of which 44% of participants used surgical masks, 78% fabric masks and 54% N95 respirators. Statistical analysis using Pearson's correlation between duration and the mean CFU/ml of fabric mask and surgical mask shows weak positive correlation (r= 0.33088 and 0.02580 respectively) with duration and N95 shows strong negative correlation (r= 0.55882), which means N95 respirators possess high filtration efficiency compared to others.

Keywords: Masks; Respiratory; infection

INTRODUCTION

The science around the use of masks by the general public to impede communicable disease transmission is advancing rapidly. The primary route of transmission of communicable diseases is likely via small respiratory droplets, and is known to be transmissible from pre-symptomatic and asymptomatic individuals[1,2]. Reducing disease spread requires two things: first, limit contacts of infected individuals via physical distancing and second, reduce the transmission by wearing masks in public. virus have been identified as the most common cause of diseases acquired within indoor environments, in particular those causing respiratory and gastrointestinal infection. Among the most common types causing respiratory infections are influenza viruses, rhinoviruses, coronaviruses, respiratory syncytial viruses and parainfluenza viruses; while those responsible for gastrointestinal infections include rotavirus, astrovirus, Norwalk-like viruses and other caliciviruses. Some of these infections are very widely spread but are not severe, such as common cold; while others are relatively more severe, like influenza[3].

A cross-sectional survey was conducted on 36 postgraduate students consisting of 4 participants from each of the 9 departments of a private dental institution of Bangalore, India, using a pretested, self- administered questionnaire. The questionnaire, consisting of 19 questions was used to assess the knowledge and practice of the participants regarding their use of the personal protective barriers. It consisted of demographic details and questions related to practice regarding the use of mouth masks, frequency of changing, its storage, exchange with colleagues, removal of mouth mask before or after a case or with gloved or ungloved hands and attempting any case without a mask. The invitro analysis involved inoculation of a section of surgical mask from the external and internal surfaces in an enrichment media for isolation of bacteria and successive isolation by spread plate method on candida agar. Chi- square test, Mann-Whitney test and Kruskal-Wallis test were used for statistical analysis. The Samples were sectioned in a laminar air flow chamber. 1cm^2 each was cut from the nose bridge of the external surface and the area adjacent to the oral cavity of the internal surface. Cut section inoculated into brain heart infusion broth (incubated at 37℃ for 24 hours at 120 rpm). Serial dilution of sample has done. Spread plated in nutrient agar (biosafety measurements), mannitol salt agar, MacConkey agar and blood agar (incubated at 37℃ for 24 hours). For the fungal culture the cut sections were pressed on surface of candida agar media for 5 minutes and removed later (incubated at 28℃ for 2-3 days). A colony counter was used to count the number of colony-forming units. The study proves that mouth mask is a major source of contamination and nosocomial infections; and following a proper infection control is necessary[4,5,6]

METHOD

The study was based on the survey and isolation of microorganisms from masks. The questionnaire was created comprising of 8 questions, and had 50 respondents. This survey was designed to standardize the data collection of used face masks, it contains two domains, i) mask wearing profile, ii) and mask wearing related knowledge and behavior.

Sample collection

A total of 12 used face masks were collected aseptically, out of which 4 were surgical masks, 4 N95 and 4 fabric masks. Used masks minimum of 1-2 hours of duration were collected in sterile zip lock pouches to culture and analyze the microbial count both inside and outside surface of each mask.

Survey design:

Masks have the potential to help in reducing the spread of communicable diseases without greatly disrupting economic activity if they are widely used. To assess the state of mask wearing, we conducted an online survey among students of SDM PG Centre, Ujire, about their recent and prospective mask wearing behavior and handling. Our survey questions on mask usage were focused on the type of mask, its usage and the treatment. All respondents are required to be SDM students, 18 years of age or older. Individual in this survey were anonymized to ensure confidentiality[4,5]

Our survey includes following questions:

1. Age: ___________

2. Place: ___________

3. Have you ever used a mask?
    - Yes
    - No
4. What type of mask did you use?
    - Fabric mask
    - Surgical mask
    - N95 mask
    - Other, please specify

5. Have you reused the mask?
    - Yes
    - No
    - Maybe

6. How many hours in a day did you wear a mask?
    - 1-2hours
    - 2-4hours
    - 4-6hours
    - More than 6 hours

7. When do you use a mask
    - Public place
    - While speaking
    - Working close proximity to others Coughing and sneezing

8. What method did you followed to disinfect the mask? Using_________________
    - Only water
    - Detergent
    - Antiseptic liquids
    - Other, please specify

Serial dilution of samples

4 aliquots of 3 different type of used masks (Fig; 01 N95 masks, surgical masks and fabric masks) were sectioned in laminar air flow chamber, $1cm^2$ of each mask sample was cut from the nose bridge of the external surface and the area adjacent to the oral cavity of the internal surface. Cut section inoculated into 9ml of sterile distilled water in a test tube and incubated at 37℃. The remaining 6 test tubes were filled with 9ml of sterile distilled water. Drawn 1ml of sample from the first test tube using a sterile pipette and transferred it into the 9ml sterile water within the second test tube to make the total volume of 10ml. It provides an initial dilution of 10^{-1}. Shaken the test tube for proper mixing. Discarded the pipette tip and used a new one. Transferred 1ml of mixture sample from the 10^{-1} dilution to the second tube and repeated the same for remaining test tubes[7,8]

Isolation of microflora from used face mask samples:

- Pipetted out 0.1ml of serially diluted sample of N95 mask from 10^{-1}, 10^{-3} and 10^{-5} dilution test tubes on to the center of the surface of 3 different agar plates.
- The sample was spread evenly over the surface of agar using sterile 'L' shaped glass spreader, carefully rotating the petri dish underneath at the same time.
- Incubated the plates at 37^0C for 24 hours.
- Repeated the same for remaining serially diluted samples.
- Counted each incubated plates, as shown in Fig: 02, Fig: 03 and Fig: 04 respectively under colony counter and calculated the CFU (colony forming unit) value of each sample[9,10].

FORMULA:

$$\text{DILUTION FACTOR} = \frac{FINAL\ VOLUME(DILUENT\ VOLUME+STOCK\ VOLUME)}{VOLUME\ OF\ STOCK\ TRANSFERRED}$$

$$\text{COLONY FORMING UNIT/ML (CFU/ML)} = \frac{NUMBER\ OF\ COLONIES \times TOTAL\ DILUTION\ FACTOR}{VOLUME\ OF\ CULTURE\ PLATED\ IN\ ML}$$

Biostatistical analysis

The results are expressed as the mean ± standard deviation. Pearson's correlation method was used to measure the strength of the linear relationship between two variables. The variables we chosen are the time duration of used masks and the mean value of cfu/ml of each sample external and internal surface. Pearson's correlation has a value between -1 to +1, with a value of -1 meaning a total negative linear correlation, 0 being no correlation, and +1 meaning a total positive correlation.

We have made use of the application such as statistical calculator, statistical solution and Microsoft word for plotting the scatter graph and correlation[111,12,13].

$$r = \frac{\sum(X - Mx)(Y - My)}{\sqrt{(\sum(X - Mx)^2 \sum(Y - My)^2)}}$$

here,

r = correlation coefficient

X= values of the X variable in a sample Y = values of the Y variable in a sample Mx = mean o the values of the X variable My = mean of the values of the Y variable

Result:

The mask sample was collected from survey respondents

- 4 N95 masks
- 4 surgical masks
- 4 fabric masks

Fabric mask sample

Surgical masks sample

N95 masks sample

Figure 1: Mask samples

Enumeration of microbial load from different mask:

Table 1: Fabric Masks

SAMPLES	INTERNAL SURFACE OF MASKS ($1cm^2$)			EXTERNAL SURFACE OF MASKS ($1cm^2$)		
	DILUTION			DILUTION		
	10^{-1}	10^{-3}	10^{-5}	10^{-1}	10^{-3}	10^{-5}
1.	112	55	7	25	9	7
2.	2	80	16	14	18	28
3.	17	18	39	176	3	19
4.	6	5	45	4	5	7

Table 2: Surgical Masks

SAMPLES	INTERNAL SURFACE OF MASKS ($1cm^2$)			EXTERNAL SURFACE OF MASKS ($1cm^2$)		
	DILUTION			DILUTION		
	10^{-1}	10^{-3}	10^{-5}	10^{-1}	10^{-3}	10^{-5}
1.	31	11	50	5	11	8
2.	50	34	26	3	6	34
3.	4	5	7	17	5	10
4.	29	34	3	10	16	3

TABLE 3: N95 Masks						
SAMPLES	INTERNAL SURFACE OF MASKS (1cm^2)			EXTERNAL SURFACE OF MASKS (1cm^2)		
	DILUTION			DILUTION		
	10^{-1}	10^{-3}	10^{-5}	10^{-1}	10^{-3}	10^{-5}
1.	85	124	91	9	90	13
2.	144	124	5	180	284	344
3.	14	6	3	5	308	3
4.	6	9	6	13	12	4

Microbial characterization from different mask:

TABLE 4: Cloth Masks

CHARACTERS	**SAMPLE 1**	**SAMPLE 2**	**SAMPLE 3**	**SAMPLE 4**
SIZE	Punctiform, moderate and large size	Small, moderate and large	Moderate and large	Moderate and large
SHAPE	Round, punctiform and irregular	Round and irregular	Round and irregular	Irregular
PIGMENTATION	White	Opaque and white	Opaque and white	Opaque and white
MARGIN	Smooth, undulate and Lobate	Smooth and undulate	Smooth, lobate, serrate and undulate	Serrate, undulate and lobate
ELEVATION	Flat and raised	Flat, raised and convex	Flat and convex	Flat
OPACITY	Opaque and transparent	Opaque and transparent	Opaque and transparent	Opaque, cloudy and transparent
TEXTURE	Viscous and brittle	Dry and viscous	Dry, shiny and mucoid	Matte finish and brittle

TABLE 5: Surgical Masks

CHARACTERS	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
SIZE	Small and large size	Small and large size	Large size	Punctiform, small and large size
SHAPE	Round and irregular	Round and irregular	irregular	Punctiform, irregular
PIGMENTATION	White and opaque	white	Milky, white and opaque	White, pink and opaque
MARGIN	Smooth and undulate	Smooth, lobate and scalloped	Undulate, serrate and lobate	Undulate, serrate and scalloped
ELEVATION	Flat and raised	Flat, raised and convex	Flat and raised	Raised and convex
OPACITY	Transparent and opaque	Transparent, translucent and opaque	opaque	Transparent and opaque
TEXTURE	Viscous and shiny	Mucoid and shiny	Dry and mucoid	Shiny, matte and viscous

TABLE 6: N95 Masks

CHARACTERS	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
SIZE	Punctiform, small and large	Punctiform, small and large	Punctiform and large	Punctiform, small and large
SHAPE	Round, punctiform and irregular	Round, punctiform and irregular	Punctiform and irregular	Punctiform and irregular
PIGMENTATION	White, pink and opaque	White and opaque	White and opaque	White, pink and opaque
MARGIN	Smooth, undulate and lobate	Smooth, scalloped and filiform	Smooth, undulate and serrate	Smooth, filiform, scalloped and lobate
ELEVATION	Flat and raised	Flat, raised and convex	Flat and raised	Flat, raised and convex
OPACITY	Transparent, translucent and opaque	Transparent and opaque	Transparent and translucent	Transparent, translucent and opaque
TEXTURE	Dry, matte and viscous	Matte, mucoid and brittle	Dry, brittle and viscous	Dry, mucoid and shiny

Colony Forming Unit/ML

Table 7: FABRIC MASKS								
SAMPLES	INTERNAL SURFACE OF MASKS (1cm^2)				EXTERNAL SURFACE OF MASKS (1cm^2)			
	CFU/ml			**Mean**	**CFU/ml**			**Mean**
	10^1	10^3	10^5		10^1	10^3	10^5	
1.	1.12×10^4	5.5×10^5	7×10^6	2520400	2.5×10^4	9×10^4	7×10^6	2371666.6
2.	2×10^2	8.0×10^5	1.6×10^7	5600066.66	1.4×10^3	1.8×10^5	2.5×10^7	8393800
3.	1.7×10^3	1.8×10^5	3.9×10^7	39181700	1.76×10^4	3×10^4	1.9×10^7	6349200
4.	6×10^2	5×10^4	4.5×10^7	15016866.66	4×10^2	5×10^3	7×10^6	2335133.33
Mean±SD = 62319033.32± 16608327.06					Mean±SD = 19449799.93±3015083.74			

Table 8: Surgical Masks								
SAMPLES	INTERNAL SURFACE OF MASKS (1cm^2)				EXTERNAL SURFACE OF MASKS (1cm^2)			
	CFU/ml			**Mean**	**CFU/ml**			**Mean**
	10^1	10^3	10^5		10^1	10^3	10^5	
1.	3.1×10^3	8×10^4	5.0×10^7	16694366.66	5×10^2	1.1×10^5	8×10^6	2703500
2.	5.0×10^3	3.4×10^5	2.6×10^7	13092.73	3×10^2	6×10^4	3.4×10^7	11353433.33
3.	4×10^2	5×10^4	7×10^6	2350133.33	1.7×10^3	5×10^4	10×10^6	3350566.66
4.	2.9×10^3	3.4×10^5	3×10^6	1114300	1.0×10^3	1.6×10^5	3×10^6	1053666.66
Mean±SD = 20171892.72 ± 7826036.17					Mean±SD = 18471166.65 ± 4594089.78			

Table 9: N95 Masks								
SAMPLE	INTERNAL SURFACE OF MASKS (1cm^2)				EXTERNAL SURFACE OF MASKS (1cm^2)			
	CFU/ml			**Mean**	**CFU/ml**			**Mean**
	10^1	10^3	10^5		10^1	10^3	10^5	
1.	8.5×10^3	1.24×10^6	9.1×10^7	307493005	9×10^2	1.3×10^5	9.0×10^5	343633.33
2.	1.44×10^4	1.24×10^6	5×10^6	2084800	1.80×10^4	2.84×10^6	3.44×10^8	115619333.33
3.	1.4×10^3	6×10^4	3×10^6	1020466.66	5×10^2	3.08×10^6	3×10^6	2026833.33
4.	6×10^2	9×10^4	6×10^6	2030200	1.3×10^3	1.2×10^4	4×10^6	1337766.66
Mean±SD = 312628471.66 ± 152891374.57					Mean±SD = 119327566.65 ± 57195800.99			

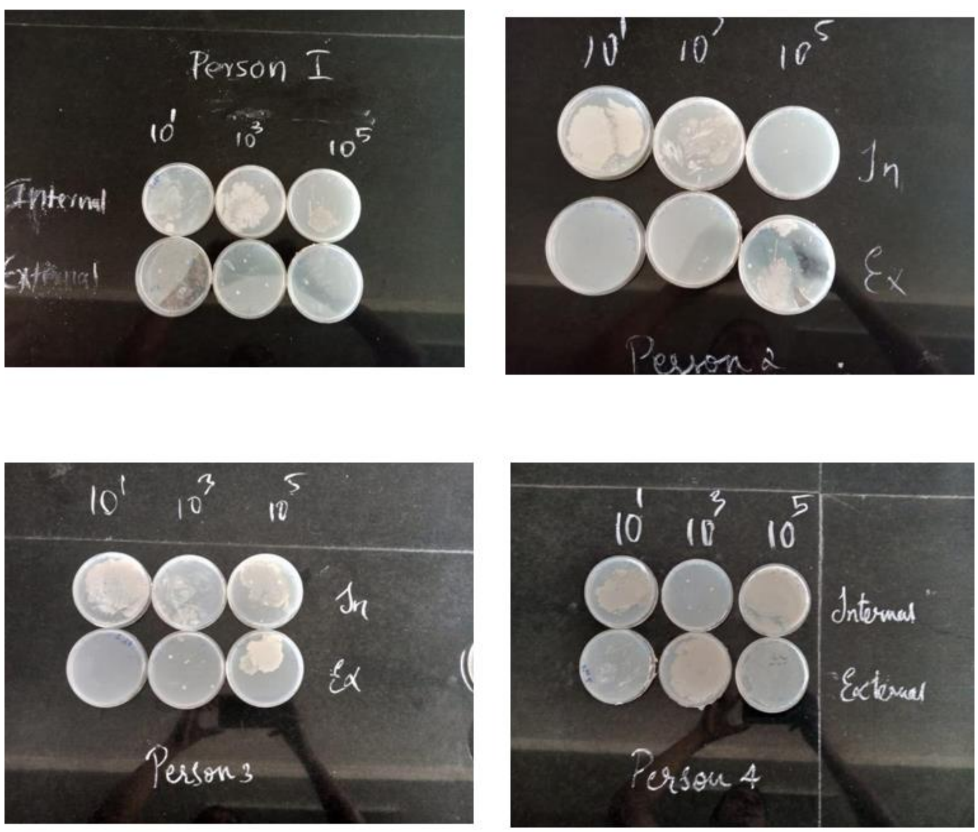


Figure 2: Spread plate of internal and external surface of surgical mask samples

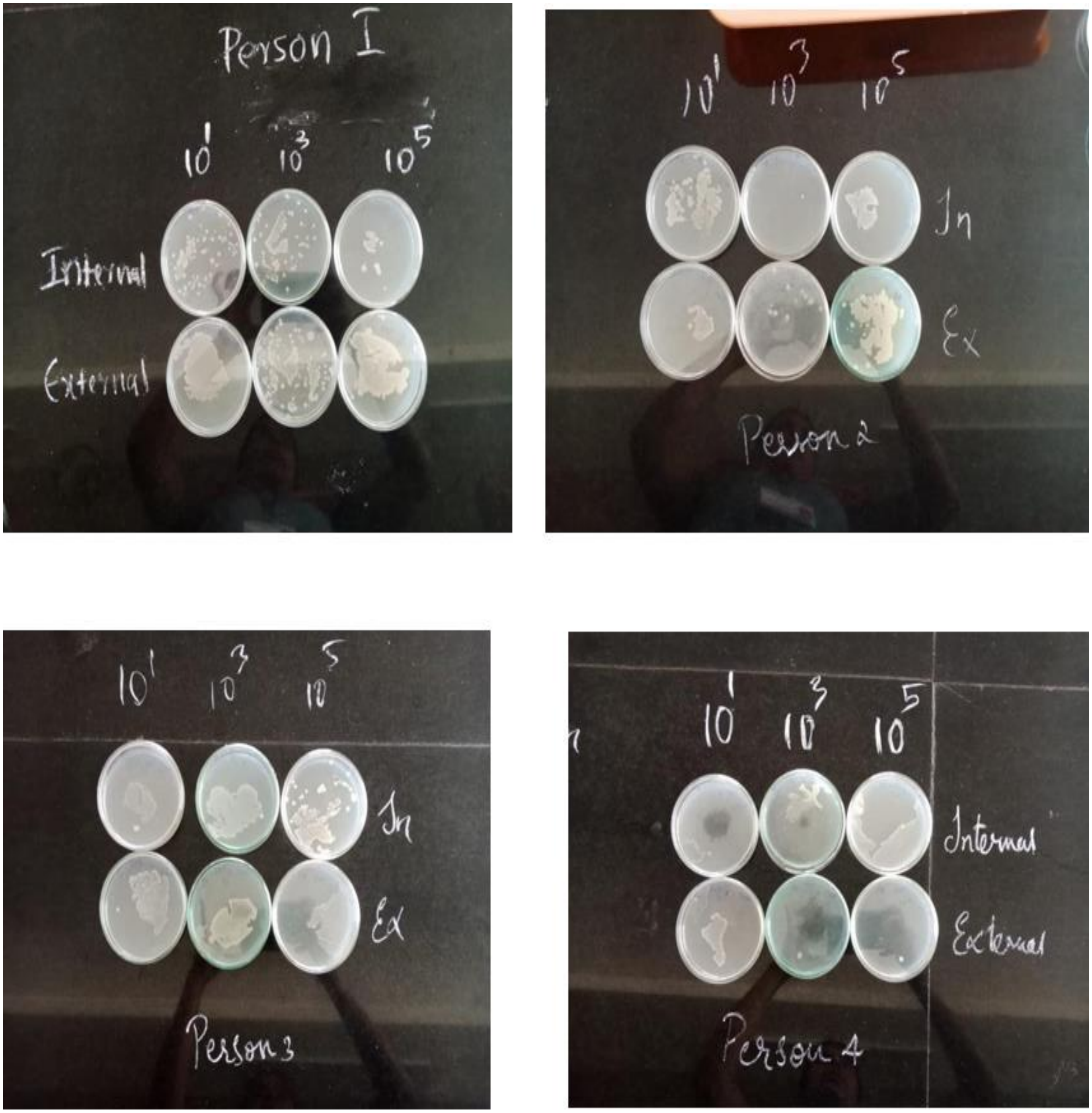


Figure 3: Spread plate of internal and external surface of fabric mask samples

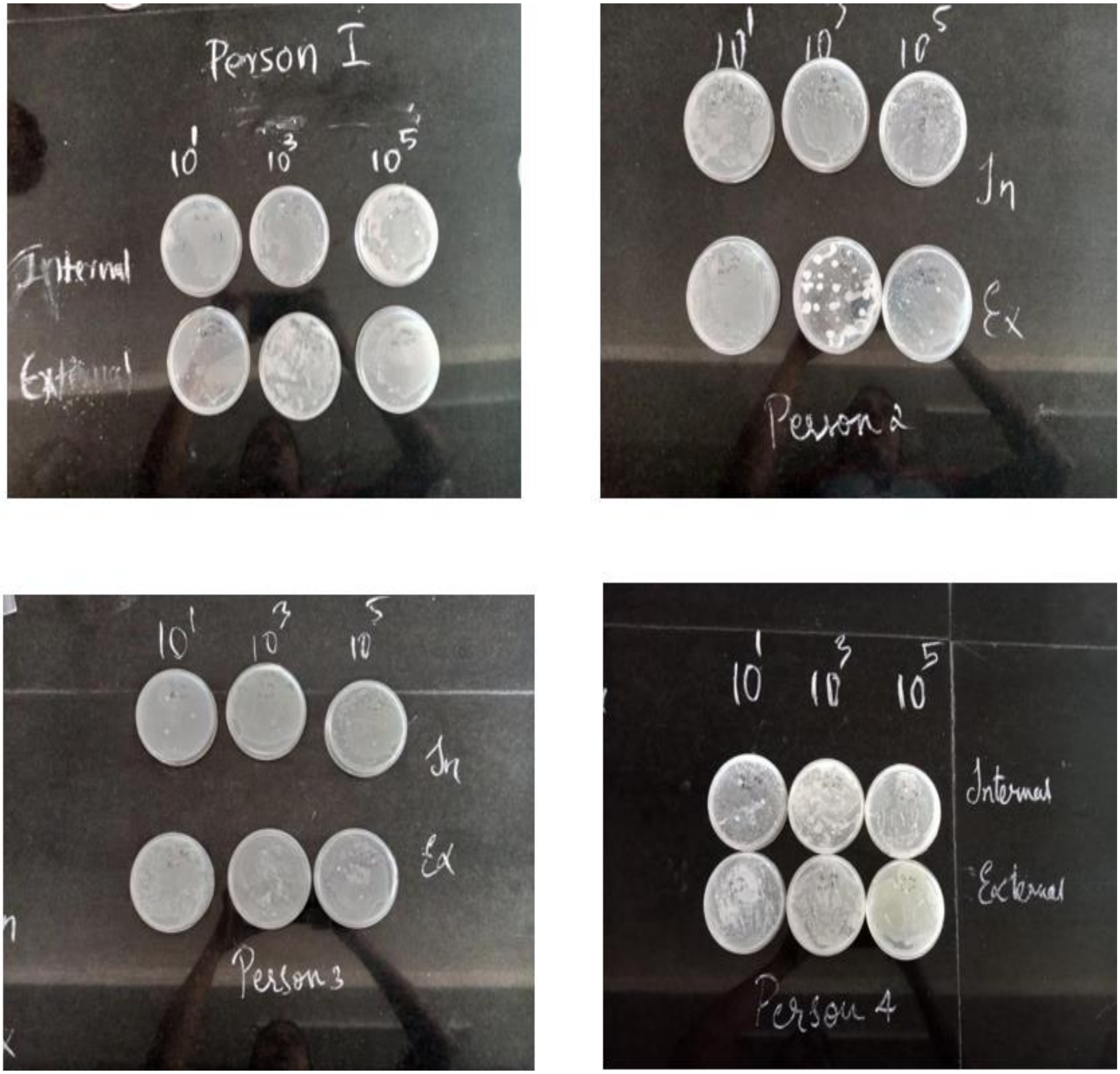
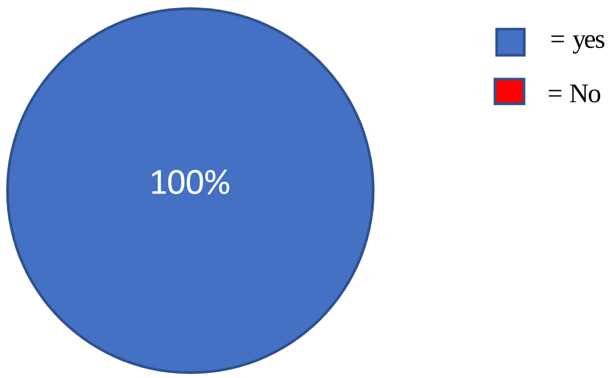


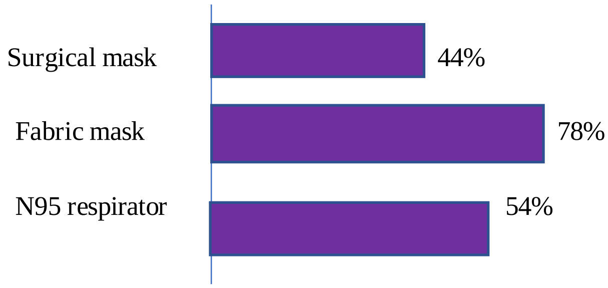
Figure 4: Spread plate of internal and external surface of N95 mask samples

Biostatistical analysis

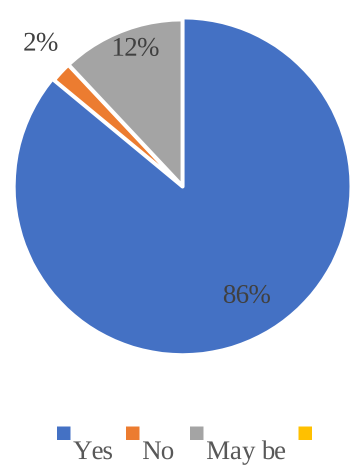
- The data obtained from survey showing that 100 % people were aware of the usage of masks and used it during covid 19 pandemic.



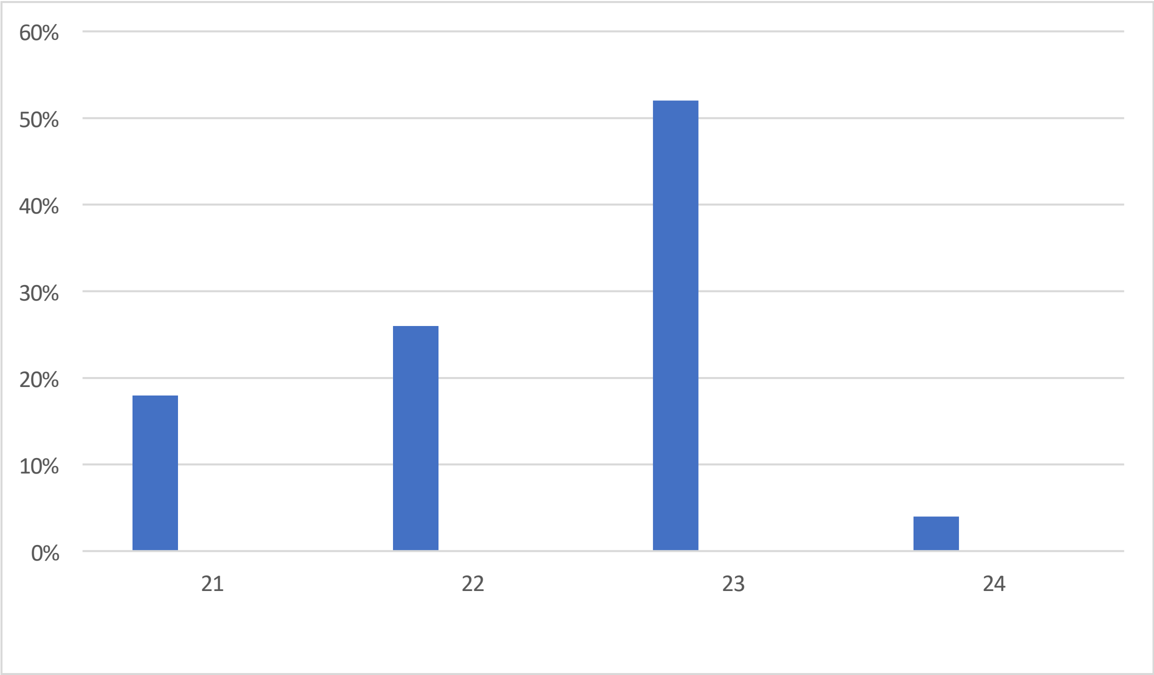
- Widely used masks are,



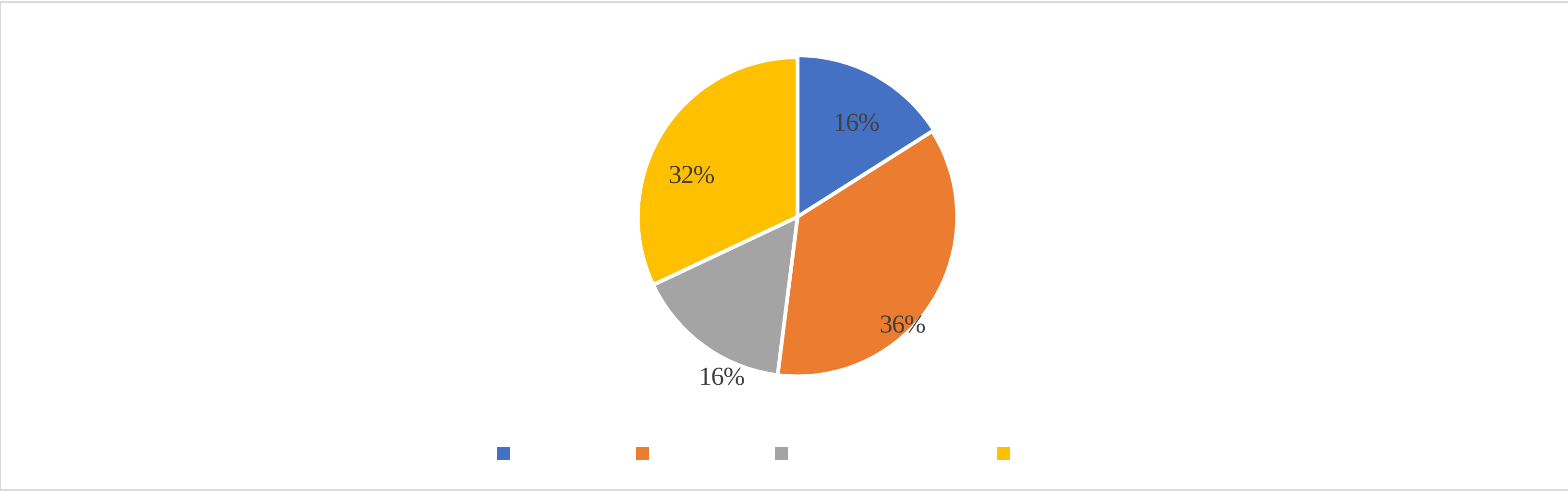
86 % people prefer reusage of masks, 2 % mentioned that reusage of masks as a strict "NO" and 12 % are not sure about the reusage of masks



- The survey respondents were the age group of 21-24 years.

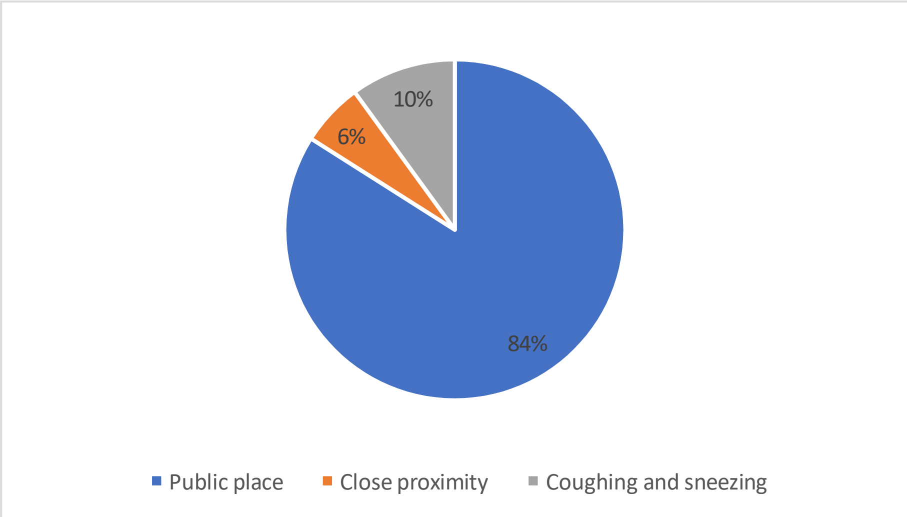


- 16 % used the same mask for more than 6 hours in a day.
  36% were used it for 4-6 hours, 32 % for 2-4 hours and 16 % for 1-2 hours.

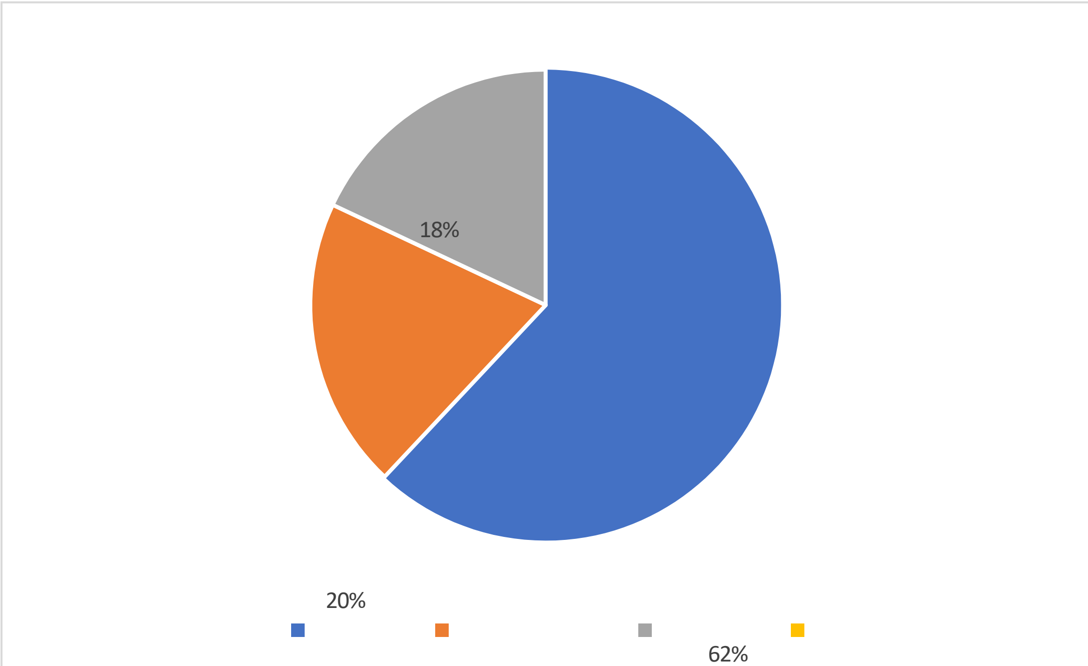


1-2 Hours 4-6 Hours More than 6 hours 2-4 Hours

- 84 % of students wore the masks in public place
  6 % of students wore when working in close proximity to other.
  10 % of them wore only during coughing and sneezing



- The method used for the disinfection of masks were, using Detergent: 62 %
  Antiseptic liquids: 20 %
  Only with water: 18 %



Pearson Product Correlation:

Table 10: Pearson product correlation of surgical masks

Sl. No	X values	Y values	X-Mx	Y-My	(□ − □□)^2	(□ − □□^{2})	(X-Mx) (Y-My)
1	2	1114300	-3	-7700704.15625	9	59300844502086.02441	23102112.46875
2	2	1053666.66000	-3	-7849437.55625	9	61613669949467.97191	23548312.66875
3	4	16694366.66000	-1	-4621037.49625	1	21353987541748.46876	4621037.49625
4	4	2703500	-1	-1827304.15625	1	3339040479448.52441	1827304.15625
5	6	13092.73000	1	28960595.84375	1	838716111625029.77441	28960595.84375
6	6	11353433.33000	1	-3871904.15625	1	14991641795186.02441	-3871904.15625
7	8	2350133.33000	3	4795762.50375	9	22999337992374.46876	14387287.51125
8	8	3350566.66000	3	-7885970.82625	9	62188535872466.10768	-23657912.47875

From the table;

Mx= mean of X values My=
mean of Y values

∑(□ − □□)(□ − □□) = 2566859.39000

∑(□ − □□)^2 = 40

∑(□ − □□)^2 = 247448552223386.19788

n = 8

r = ∑(□−□□)(□−□□) / √(∑(□−□□)^2 ∑(□−□□)^2)

$$r = \frac{2566859.39000}{\sqrt{(40)(24744552223386.19788)}}$$

r **= 0.02580**

There is a weak positive correlation between X and Y. A weak positive correlation indicates that, although both variables tend to go up in response to one another, the relationship is not very strong.

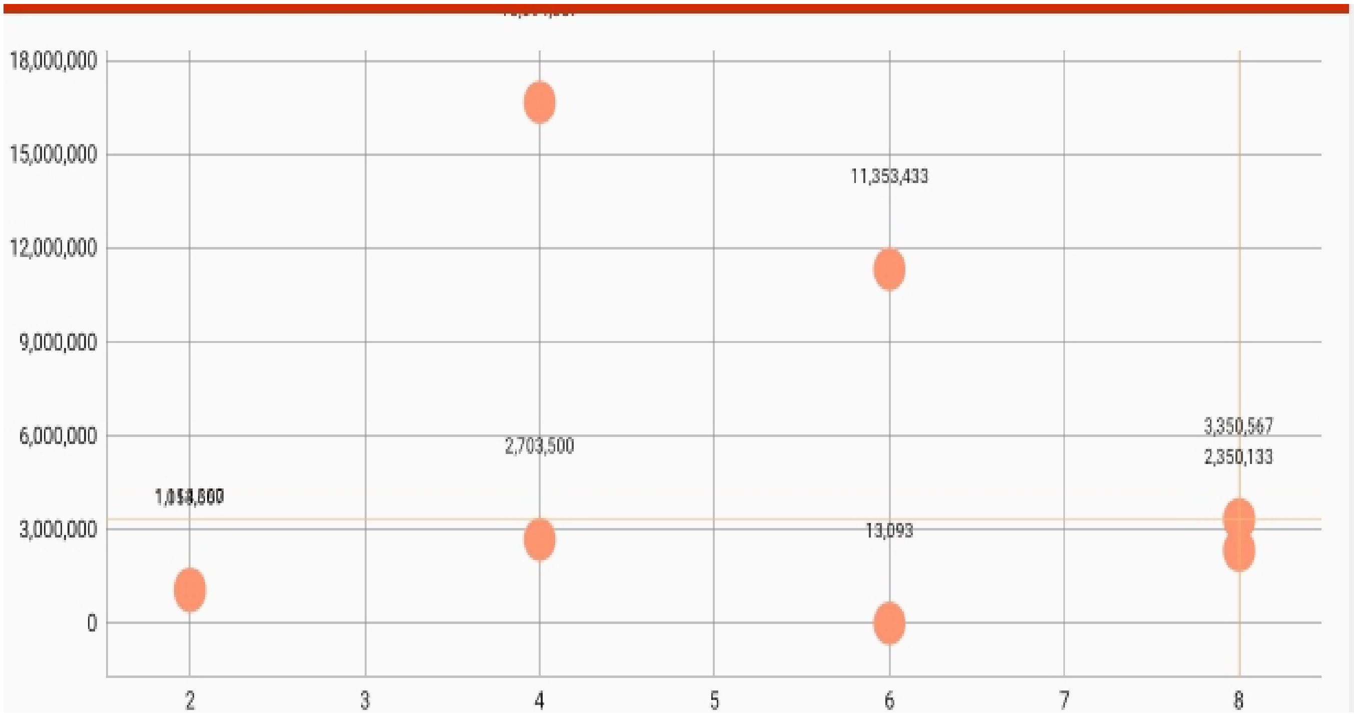


Figure 5: Pearson's correlation scattered plot of surgical mask showing weak positive correlation between duration of usage and mean of CFU/ml of internal and external surface of masks.

Table 11: Pearson product correlation of fabric masks

Sl. No	X-value	Y-value	X-Mx	Y-My	(□ − □□)^2	(□ − □□^{2})	(X-Mx) (Y-My)
1	2	2520400	-3	-7700704.16375	9	59300844617596.58681	23102112.49125
2	2	2371666.60000	-3	-7849437.50375	9	61613669125277.03126	23548312.51125
3	4	5600066.66000	-1	-4621037.50375	1	21353987611064.03126	4621037.50375
4	4	8393800	-1	-1827304.16375	1	3339040506858.08681	1827304.16375
5	6	39181700	1	28960595.83625	1	838716111190620.83681	28960595.83625
6	6	6349200	1	-3871904.16375	1	14991641853264.58681	-3871904.16375
7	8	15016866.66000	3	4795762.49625	9	22999337920438.03126	14387287.48875
8	8	2335133.33000	3	-788597083375	9	62188535990755.67013	-23657912.50125

From the table;

Mx = mean of X values My =

mean of y values

$\sum(X - M_x)(Y - M_y) =$ 68916833.51000

$\sum(X - M_x)^2 =$ 40

$\sum(Y - M_y)^2 =$ 108450316975807.36478

n = 8

$$r = \frac{\sum(X-M_x)(Y-M_y)}{\sqrt{(\sum(X-M_x)^2 \ \sum(Y-M_y)^2)}}$$

$$r = \frac{68916833.51000}{\sqrt{((40)(1084503169757807.36478))}}$$

= 0.33088

There is a weak positive correlation between X and Y. A weak positive correlation indicates that, although both variables tend to go up in response to one another, the relationship is not very strong.

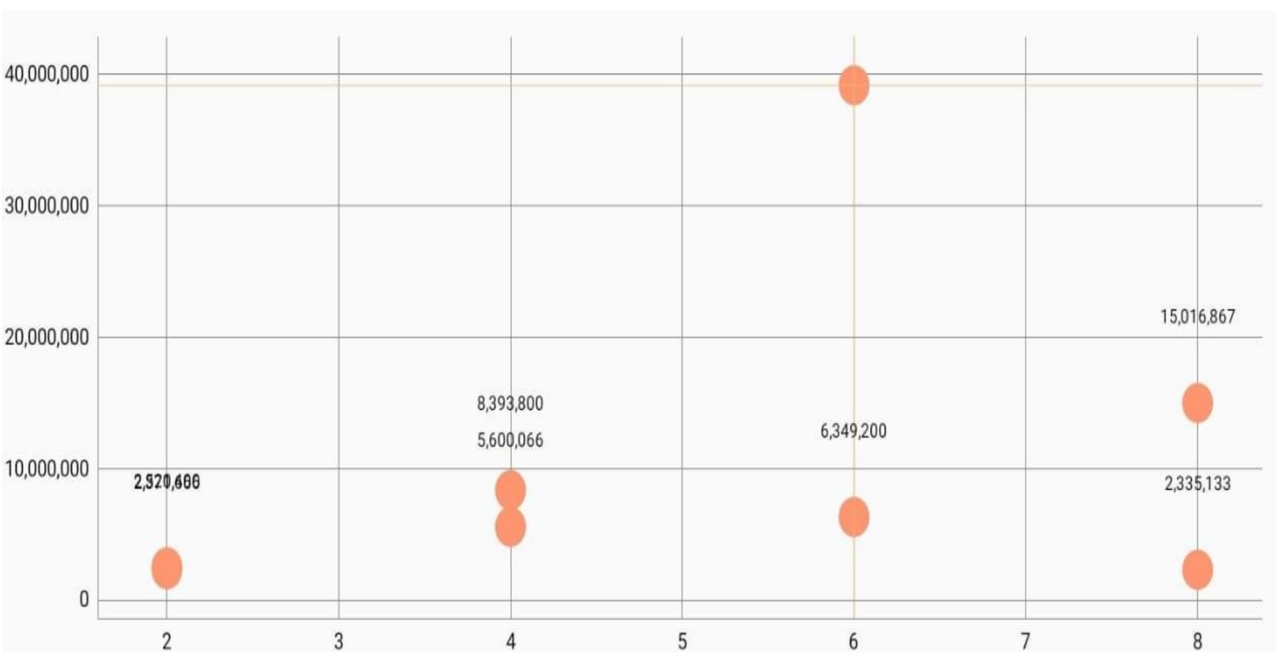


Figure 6: Pearson's correlation scattered plot of fabric mask showing weak positive correlation between duration of usage and mean of CFU/ml of internal and external surface of masks.

Table 12: Pearson product correlation of N95 respirators

Sl. No	X value	Y value	X-Mx	Y-My	$(X-Mx)^2$	$(Y-My)^2$	(X-Mx) (Y-My)
1	2	307493005	-3	253498500.21125	9	642614896093531 16.29462	-760495500.63375
2	2	343633.33000	-3	-5365087145875	9	2878416008283315.35295	160952614.37625
3	4	2084800	-1	-51909704.78875	1	2694617451255174.68212	51909704.78875
4	4	115619333.33000	-1	61624828.54125	1	3797619492738460.60295	-61624828.54125
5	6	1020466.66000	1	-52974038.12875	1	2806248715666258.80157	-52974038.12875
6	6	2026833.33000	1	-51967671.45875	1	2700638876844579.35295	-51967671.45875
7	8	2030200	3	-51964304.78875	9	2700288972178106.18212	-155892914.36625
8	8	1337766.66000	3	-52656738.12875	9	2772732070359754.05157	-157970214.38625

From the table;

Mx = mean of X values My = mean of y values

$\sum(\square - \square\square)(\square - \square\square) =$ -1028062848.35000

$\sum(\square - \square\square)^2 =$ 40

$\sum(\square - \square\square)^2 =$ 84612051196678765.32088

n = 8

$r = \frac{\sum(\square-\square\square)(\square-\square\square)}{}$

$$\sqrt{(\sum(□-□□)^2 \ \sum(□-□□)^2)}$$

$$r = \frac{-1028062848.35000}{\sqrt{((40)(84612051196678765.32088))}}$$

$$r = -\mathbf{0.5588}$$

There is a strong negative correlation between X and Y. A strong negative correlation indicates a strong connection between the two variables, but that one goes up whenever the other one goes down

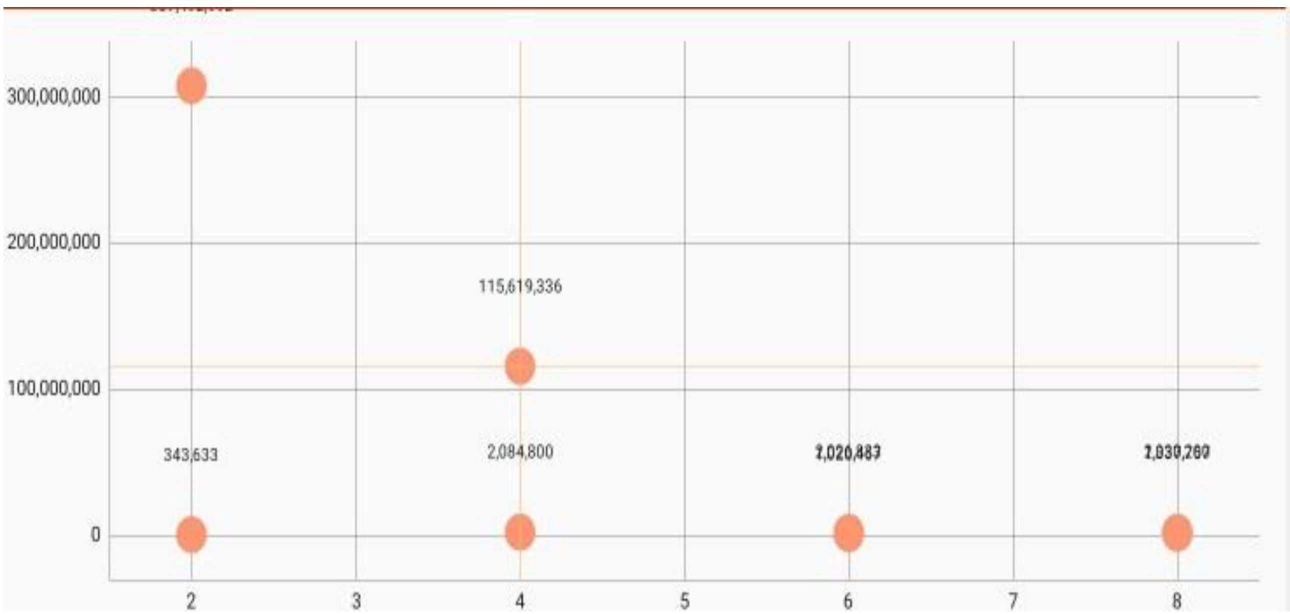


Figure 7: Pearson's correlation scattered plot of N95 respirator showing strong negative correlation between duration of usage and mean of CFU/ml of internal and external surface of masks

DISCUSSION

In the present study, a cross-sectional questionnaire revealed that 86% of participants prefer reusage of masks, 16% participants used the same mask more than 6hours in a day, out of which 44% of participants used surgical masks, 78% fabric masks and 54% N95 respirators. Maryam Amour et al. conducted an invitro experiment to determine filtration efficiency for selected face masks to bacteria with a size smaller than SARS-CoV-2 respiratory droplet. Bacterial filtration efficiency (BFE) was determined as the proportions of colony forming units (cfu) between the test and control mask. The selected face masks had BFE of 100% and > 99% for medical and double layer cotton cloth mask, respectively. Their study supports the use of cotton cloth (at least double layer) face coverings in public settings where other social distancing measures are difficult to maintain to prevent the spread of infection from the wearer. In our research, Pearson's correlation between duration and the mean CFU/ml of fabric mask and surgical mask shows weak positive correlation (r= 0.33088 and 0.02580 respectively) with duration and N95 shows strong negative correlation (r= 0.55882), as indicated in Fig:05, Fig:06 and Fig 07 respectively, which means N95 respirators possess high filtration efficiency compared to others.

According to Morawska. L, 2006, reducing disease spread requires two things, first, limit contact of infected individuals by physical distancing and second, reduce the transmission by wearing mask in public. Even though 84% of participants of our survey wore the mask in public place, 6% wore only in close proximity to others and 10% of them wore only during coughing and sneezing, this might be one of the reasons for the contamination of masks, which results the spread of communicable diseases.

Liu Zhiqing et.al 2018, study results demonstrated that the contamination of the Surgical mask (SM)surface worsen with wearing extension. Meanwhile, a high variation existed among different surgeons. In addition, the mean of CFUs isolated from the first layer mask (close to the face) was higher compared to the second layer (far from the face) in double layered SMs. Our study result also shows microbial contamination on the surface of masks worsen with wearing extension, and also shows that the microbial load in the surface of masks not only depend on duration but also depends on wearer, hygienic practices and adequate usage.

P Luksamijarulkul et al. concluded that high bacterial contamination on outside area of the used mask and it showed a significant correlation with microbial air quality of working wards. In our study result internal surface of mask contain more microbial count than external surface.

CONCLUSION

Based on our research we mainly draw three conclusions; (1) The internal surface of each sampled masks contains more microorganisms when compared to external surface; (2) The microbial load in the surface of masks not only depend on duration but also depends on wearer, hygienic practices and adequate usage; thus, we recommend proper usage of masks based on current WHO guidance and (3) high filtration efficient masks, such as N95 respirators could be an effective measure in reducing communicable diseases.

There were several limitations in this study that should be noted. First, the internal and the external surface of mask was the region of interest; however, the sampling operation could increase the risk of cross contamination. We carefully collected the used masks and tried our best to reduce the risk of cross contamination. Meanwhile, we strictly adhere to the protocol of sampling operation to ensure the consistency and reliability. In addition, we also realized that there are likely numerous brands of masks that are made of different materials. Some of these might perform better than others in preventing microbial shed. Comparing specific brands of masks was beyond the scope of this study and could be considered for additional studies.

REFERENCE

1. Enalgbe AA, Akpoka OA, Irodi CC. Bacterial contaminations of used face masks collected from different clinical sections in a university teaching hospital during Covid-19 pandemic crises in Nigeria. Scicell. Bacterial empire: 2021;4(8-10).
2. Rogers KB. An investigation in to the efficiency of disposable face masks. J Clin pathol: 1980;33(1086-1091).
3. Zhiqing L, Yongyun C, Wenxiang C, Mengning Y, Yuanqing M, et al. Surgical masks as source of bacterial contamination during operative procedures. Science direct. Journal of orthopedic translation: 2018;14(57-62).
4. Wesley Y, Kennedy Ng, Nigel fong JM. Assessment of proficiency of N95 mask donning among the general public in Singapore. Jama network open: 2020;03(5).
5. WHO. Mask used in the context of Covid-19, interim guidance, one December 2020.
6. WHO. Technical specifications of personal protective equipment for Covid-19, interim guidance, 2020.
7. Ueki H, Furusawa Y, Iwatsuki-Horimotok, Imai N. Effectiveness of face masks in preventing airborne transmission of SARS-CoV-2. American society for microbiology. Msphere: 2020;5.
8. Sagar A. Nutrient agar: composition, preparation and uses. 2022.
9. Science assist. Standard operating procedure: preparing agar plates. Studylib.net. 2014.
10. Chemwatch gold. Long safety data sheet: Nutrient agar 2014.
11. Blaize JF, Sutere, Corbo CP. serial dilutions and plating: microbial enumeration. Jove.com.
12. Sanders ER. Aseptic laboratory technic: plating methods. Jove 63: e3063. 2012.
13. Ben-David A, Davidson CE. Estimation method for serial dilution experiments. Journal of microbiological methods 2014;107:214-221.
14. Ford CR, Peterson DE, Mitchell CR. An appraisal of the role of surgical face masks Am J Surge 1962;113:787-790.
15. Greene VW, Vesley D. Methods for evaluating effectiveness of surgical masks. J Bacteriol 1962;83:663-666.
16. Rohan S, Garg K. Is safe guard compromised? Surgical mouth mask harboring hazardous microorganisms in dental practice. Journal of family medicine and primary care. Medknow publications 2020);2(759-763).
17. Monalisa, Aruna CN, Padma KB. Microbial contamination of the mouth mask used by post-graduate students in a private dental institution: an invitro study. IOSR J Dent Med Sci. 2017;16:61-70.
18. Bio-resource. CFU: Colony forming unit & calculation. 2011.
19. Types of masks and respirator CDC.

20. Young C, Bacer. Types of masks and how effective they are. Healthgrades.com. 2022.
21. FDA, N95 respirators, surgical masks, face masks and barrier face coverings. (2022).
22. Chen Y. Spatial autocorrelation approaches to testing residuals from least squares regression. Plos-01. 2016.
23. Mahoney J. Beyond correlation analysis: recent innovations in theory and method. Sociological forum JSTOR. 2001;(575-593)
24. Godfrey KR. Correlation methods. Science direct. Automatica. 1980;16(527-534)
25. Ade K. Research methodology module-06. 2018.
26. Senthilnathan S. Usefulness of correlation analysis. ResearchGate.net. 2019.
27. Yadav S. Correlation analysis in biological studies. Journal of the practice of cardiovascular sciences. 2018.
28. Data correlation Statistical solutions.