Article **Journal of Health Sciences**

December 2022, Vol.9 No.27 18-23

Isolation and identification of microorganism present on the external and internal surfaces of face masks

Aislamiento e identificación de microorganismos presentes en superficies externa e interna de cubrebocas

BASTO-MIJANGOS, Harold N.†*, CAAMAL-LEY, Ángel D., PUC-FRANCO, Miguel A. and VARGAS-GONZALEZ, Alberto

Universidad Autónoma de Yucatán. Centro de Investigaciones Regionales "Dr. Hideyo Noguchi. Av. Itzáes, Centro, 97000 Mérida, Yuc.

ID 1st Author: *Harold N., Basto-Mijangos* / **ORC ID:** 0000-0003-3618-1360, **CVU CONACYT ID**: 1090296

ID 1st Co-author: *Angel D., Caamal-Ley /* **ORC ID:** 0000-0002-9486-6731, **CVU CONACYT ID**: 103523

ID 2nd Co-author: *Miguel A., Puc-Franco* / **ORC ID:** 0000-0002-6016-8716, **CVU CONACYT ID:** 296453

ID 3rd Co-author: *Alberto, Vargas-Gonzalez* / **ORC ID:** 0000-0001-8761-3990**, CVU CONACYT ID:** 60894

DOI: 10.35429/JOHS.2022.27.9.18.23 Received July 20, 2022; Accepted December 30, 2022

Abstract

Continuous use of the face mask without replacement provides humidity and temperature conditions conducive to the development of multiple disease-causing microorganisms. The objective of the study is to identify, through phenotypic tests, the presence of bacteria in face masks in order to know if there are potentially pathogenic microorganisms. Likewise, differentiate the amount of CFU / mL depending on the sampled section. For this, a sample was taken with sterile swabs moistened with isotonic saline solution to 20 masks (internal and external part separately). The CFU / mL count of 15 face masks was made by sowing them on nutrient agar. In addition, all the samples were seeded on blood agar and the bacteria were subsequently identified using conventional biochemical tests. Among the isolated microorganisms are pathobionts such as *Staphylococcus epidermidis* and *Staphylococcus aureus*, as well as opportunistic microorganisms such as *Escherichia coli*, *Pseudomonas spp*. and *Hafnia alvei*. A significant difference was found in the amount of CFU / mL recovered from the internal and external part. This suggests that the continuous use of a mask can create adequate conditions for the proliferation and storage of pathobiont or opportunistic bacterial species.

Face mask, Opportunistic, Pathogen

Resumen

El uso continuo del cubrebocas sin recambio provee de condiciones de humedad y temperatura propicios para el desarrollo de múltiples microorganismos causantes de enfermedades. El objetivo del estudio consiste en identificar mediante pruebas fenotípicas la presencia de bacterias en cubrebocas con la finalidad de conocer si existe microorganismos potencialmente patógenos. Así mismo, diferenciar la cantidad de UFC/mL en función de la sección muestreada. Para esto se tomó una muestra con hisopos estériles humedecidos con solución salina isotónica a 20 cubrebocas (parte interna y externa por separado). Se realizó el conteo de UFC/mL de 15 cubrebocas mediante su siembra en agar nutritivo. Además, la totalidad de las muestras fueron sembradas en agar sangre y posteriormente se identificaron las bacterias mediante pruebas bioquímicas convencionales. Entre los microorganismos aislados se encuentran patobiontes como *Staphylococcus epidermidis* y *Staphylococcus aureus*, así como microorganismos oportunistas tales como *Escherichia coli*, *Pseudomonas* spp. y *Hafnia alvei.* Se encontró diferencia significativa en la cantidad de UFC/mL recuperados de la parte interna y externa. Lo anterior sugiere que el uso continuo de un cubrebocas puede crear condiciones adecuadas para la proliferación y almacenamiento de especies bacterianas patobiontes u oportunistas

Cubrebocas, Oportunista, Patógeno

Citation: BASTO-MIJANGOS, Harold N., CAAMAL-LEY, Ángel D., PUC-FRANCO, Miguel A. and VARGAS-GONZALEZ, Alberto. Isolation and identification of microorganism present on the external and internal surfaces of face masks. Journal of Health Sciences. 2022. 9-27:18-23.

* Correspondence to Author (E-mail: haroldnoe14@hotmail.com)

† Researcher contributing as First Author

Introduction

With the emergence of the SARS-COV2 pandemic, the use of face masks has spread as a preventive measure to prevent the spread of microdroplets with infectious agents. These particles are retained in the masks because they are larger than the pore size. (Delanghe *et al*., 2021)

However, the continuous use of the mask provides humidity and temperature conditions to the development of multiple microorganisms from the mouth (*Streptococcus* spp., *Neisseria* spp., *etc.*) and from the skin surface (*Staphylococcus* spp., *etc*.), and even acquires an even greater dimension if one considers the multiple times in which the hands touch the face mask for its adjustment, thus transferring microbes from our environment. (Aruna *et al*., 2017; Delanghe *et al*., 2021; Procop *et al.*, 2017)

Delanghe *et al*. (2021), described isolates of the genera: *Acinetobacter* spp., *Neisseria* spp., *Streptococcus* spp., *Staphylococcus* spp., *Sphingomonas* spp. and *Roseomonas* spp., in face masks with 4 hours of use; In addition, a significant difference was found between the initial and post-use bacterial load.

Luksamijarulkul *et al.* (2014), carried out a test on the face masks of hospital workers in order to determine the types of microorganisms; highlight the presence of *Staphylococcus* spp., Pseudomonas spp. and species of filamentous fungi. In addition, we found significant differences between the CFU/mL obtained from the internal part of the mask and the external part (higher mean).

Aruna *et al.* (2017), described the presence of multiple pathogens in a sample of mouth covers: *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Enterobacter* spp. and *Staphylococcus* aureus. These are the cause of a wide variety of respiratory, skin, genitourinary and gastrointestinal diseases.

The general population is unaware of the impact of microorganisms as the cause of infectious diseases. Therefore, the use of face masks continuously and without replacement is frequent, becoming a fomite of potentially harmful bacteria.

The aim of the study was to identify the presence of bacteria in a sample of face masks through phenotypic tests in order to determine if there are potentially pathogenic microorganisms. Likewise, differentiate the amount of CFU/mL depending on the sampled section (internal and external part).

Methodology

The diversity of bacterial microorganisms presents on the inside and outside of the volunteers' face masks was identified.

Sampling

Convenience samples were taken from 20 face masks donated by volunteer researchers during the confinement period due to the COVID 19 pandemic in the months of February to April 2021; these had 1-8 days of use. For each sampling, two sterile swabs moistened with isotonic saline solution were used. One was rotated on the inner surface of the mask and the other on the outer, trying to cover the entire length.

Subsequently, each swab was placed in a 5 mL sterile glass tube containing 1 mL of sterile isotonic saline solution and proceeded in a maximum period of 1 hour.

Microbiological culture

The content of the swab was homogenized in the volume of saline solution. To count the CFU/mL in 15 masks, 10 µL was dispensed on a Nutrient agar plate (MCDLab, Mexico) and later, it was streaked throughout the plate. It was incubated in a bacteriological oven (Riossa series: ECML. México®), at 37°C for 24 hours.

On the other hand, with each swab of the 20 face masks, a blood agar plate (MCDLab, Mexico) was inoculated and streaked with a sterile round bacteriological loop using the pentagon technique. It was incubated at 37°C for 24 hours.

Colonial count

At the end of the incubation time, the CFU of each plate was counted and multiplied by 100 to obtain the corresponding CFU/mL.

Identification of microorganisms

From the cultures with development, a descriptive analysis was carried out to identify the morphological differences of the colonies and the Gram staining of each colony was carried out, as well as the catalase and oxidase tests.

Subsequently, based on the preliminary data, Gram-negative bacilli and cocobacilli were reseeded on McConkey agar (MCDLab, Mexico), and biochemical tests were performed: citrate, MIO, LIA, urea, and KIA to identify Enterobacterales and other microorganisms.

In addition, resistance tests to Novobiocin and coagulase (free and bound) were carried out for the identification of staphylococci and Schaeffer–Fulton staining for the observation of spores and Gram-positive bacilli.

After 24 hours of incubation at 37°C of the biochemical tests, the analysis of the results was carried out based on the tables of biochemical reactions of bacterial species proposed by both Procop *et a*l. (2017), as well as Cowan & Steel (2003).

Statistic analysis

The CFU/mL values were grouped into sets and compared using the Mann-Whitney U test, Student's t test, and Pearson's correlation using the Past4.05 statistical software. An $\alpha = 0.05$ was used.

Results

The CFUs of the internal and external part of 15 face masks were counted, the data collected is summarized in Table 1.

The comparison of the data (CFU/mL values other than zero), grouped into two sets (internal and external), using the Mann-Whitney U test revealed that there is a significant difference (p= 0.018) between the medians of the internal group ($\tilde{X}$=800) and external group ($\tilde{X}$=50), the first being the largest.

CODE	UFC/10µL	UFC/mL	Type	Days of use
CB1E	0	0	Reusable	1
CB1I	0	0	Reusable	1
CB2E	0	0	Reusable	5
CB2I	1	100	Reusable	5
CB3E	0	0	Disposable	8
CB3I	1	100	Disposable	8
CB4E	0	0	Disposable	1
CB4I	3	300	Disposable	1
CB5E	0	0	Disposable	1
CB5I	63	6300	Disposable	1
CB6E	0	0	Disposable	> 1
CB6I	16	1600	Disposable	> 1
CB7E	2	200	Disposable	1
CB7I	5	500	Disposable	1
CB8E	0	0	Disposable	1
CB8I	83	8300	Disposable	1
CB9I	8	800	Disposable	1
CB10E	7	700	Disposable	1
CB10I	24	2400	Disposable	1
CB11E	2	200	Disposable	1
CB11I	6	600	Disposable	1
CB12E	75	7500	Reusable	1
CB12I	47	4700	Reusable	1
CB13E	1	100	Reusable	1
CB13I	1	100	Reusable	1
CB14E	41	4100	Reusable	2
CB14I	13	1300	Reusable	2
CB15E	2	200	Disposable	1
CB15I	20	2000	Disposable	1

Table 1 Summary of data from 15 face masks sampled for CFU counting

In order to find out if there is a difference between the isolated CFU/ml according to the type of mask, the data from the internal part (values of CFU/mL other than zero) were grouped into two sets (disposable and reusable). From these, it was compared using the Student's t test and it was found that there is no significant difference (p=0.1099) between the CFU/mL means in both groups.

On the other hand, data from the external part (non-zero CFU/mL values) were collected in two sets (Disposable and reusable). These were compared using the Mann-Whitney U test and it was found that there is no significant difference (p=0.4742) between the CFU/mL medians in both groups. With the intention of knowing if there is a correlation between the days of use and the amount of CFU/mL, the Pearson correlation test was used. The data (CFU/mL values other than zero and exact number of days) was divided into two groups (internal and external), then the statistical test was applied for both the internal group (p=0.298) and the external group. (p=0.656), no statistically significant correlation was obtained. Therefore, there is no correlation between days of use and isolated CFU/ml.

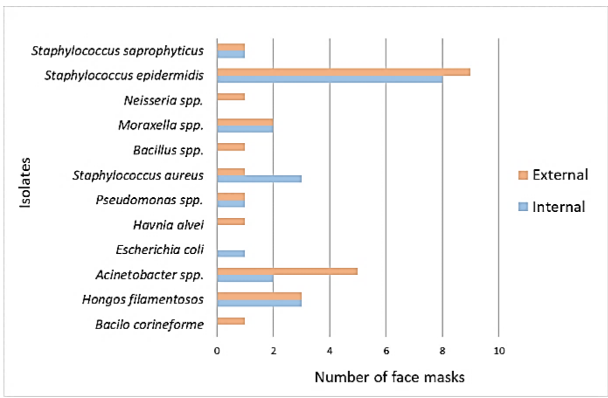
ISSN- 2410-3551

On the other hand, a total of 20 face masks were planted in culture media in order to identify the existing bacteria through phenotypic tests (Table 2). Of these, a higher percentage of *Staphylococcus epidermidis* and *Acinetobacter* spp.

Microorganism	Percentage
Staphylococcus epidermidis	73.68%
Acinetobacter spp.	31.58%
Hongos filamentosos	26.32%
Staphylococcus aureus	21.05%
Moraxella spp.	15.79%
Pseudomonas spp.	10.53%
Staphylococcus saprophyticus	10.53%
Bacillus spp.	5.26%
Bacilo corineforme	5.26%
Escherichia coli	5.26%
Havnia alvei	5.26%
Neisseria spp.	5.26%

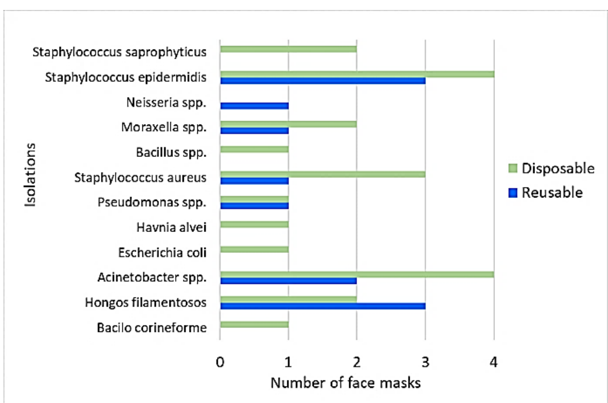
Table 2 Percentage of bacteria isolates based on the total number of face masks

In the same way, the isolates were grouped according to the recovery site. These are reflected in graphic 1.



Graphic 1 Isolations of microorganisms depending on the recovery site (internal or external part)

The data was analyzed based on the particular type of mask (disposable or reusable) where it was recovered (Graphic 2).



Graphic 2 Isolations of microorganisms depending on the type of face mask (disposable or reusable)

Discussions

Regarding the performance of the colonial count, in table 1 CFU values equal to 0 are observed, this does not necessarily indicate the absence of microorganisms in said mouth covers, but rather that the real CFUs are less than 1 CFU per 10μL.

The statistical analysis reflected significant differences in the amount of CFU/mL recovered from the internal part (higher median), with respect to the external part of the face mask. This is within what is expected as it is a retention mechanism for fluids and aerosols; however, this favors the accumulation of microorganisms in close contact with the skin. In relation to the above, the bacterial organisms with the highest percentage of isolates were *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Acinetobacter* spp.

Staphylococcus epidermidis is known as part of the skin microbiota together *Staphylococcus aureus*, Delanghe *et al*. (2021), describe these two organisms as pathobionts capable of causing atopic dermatitis and acne vulgaris. Foo *et al.* (2006), point out that the main adverse reaction caused by the use of disposable face masks (N95) was the appearance of acne that may have its origin in temperature and humidity conditions; these conditions are described as conducive to bacterial infections, as also described by Saavedra *et al.*, (2011).

Likewise, Hua *et al*. (2020), refer to the friction caused by the face mask and the decrease in pH (an important microbial regulator), as factors that contribute to altering the function of the skin barrier.

The isolation of *Acinetobacter* spp. It is within expectations because it is recognized as part of the normal microbiota of the skin. On the other hand, in this study the isolation of Streptococcus spp. which may be due to its low quantity in the samples in relation to other competing microorganisms. (Delanghe *et al*., 2021)

In the present study, *Pseudomonas* spp. in 10.53% of the face masks; around this Luksamijarulkul *et al*. (2014), also described the recovery of *Pseudomonas* spp in mask samples (37%). This genus of bacteria is implicated in a diversity of infections, especially associated with susceptible people; likewise, the species of this genus tend to present resistance to antibiotic treatments with relative frequency. (Bennett *et al*., 2017; Estepa, 2014).

On the other hand, the greatest diversity of isolates was recovered from the external part of the mask, these microorganisms from the environment can reach the mask through the hands. A recovered species was *Hafnia alvei*, which is known to be an opportunistic pathogen that causes cystitis. (Gund *et al*., 2021; Orrego *et al*., 2014).

Regarding the internal part of the mask, in one of the isolates the presence of *Escherichia coli* was found, this microorganism has been described as causing gastro-intestinal infections as well as urinary infections. (Orrego *et al*., 2014).

Similarly, the development of species of filamentous fungi was observed in 26.32% of face masks. these are retained in the mouth covers from the environment. Aruna *et al*. (2017), also refer to isolates of filamentous fungi from samples of mouth covers which come from the environment. Because these microorganisms are ubiquitous, their isolation from face masks is expected thanks to the ease with which the spores are retained in them.

The statistical comparison of the CFU/mL of the microorganisms isolated from different sites (internal and external separately) was made according to the type of face mask, but no significant differences were found. This indicates that the means or medians of CFU/mL recovered from both the internal and external parts are the same regardless of the type of mask. However, a greater variety of isolates is observed in those of the disposable type, this may be due to the less care given to them as they are for single use.

On the other hand, no correlation was found between the CFU/mL and the days of use of the mask; however, the original sample was small, so it cannot be ruled out that this type of interaction may exist in a larger sample.

Face masks are protective equipment that prevent the transmission of infectious diseases; however, they also provide humidity and temperature conditions that make it a favorable environment for the development of potentially pathogenic bacterial species. Delanghe *et al.* (2021), point out the accumulation of pathobiont bacteria in the face mask after 4 hours of use and recommend discarding disposable face masks after use.; In addition, they refer to the possibility of causing dysbiosis and subsequent association with acne due to prolonged use of it (greater than 4 hours).

Acknowledgement

We thank Ana Guadalupe Basto Mijangos for her support in translating this article into English.

Conclusions

The face mask is a useful tool in the current situation of the SARS-COV2 pandemic, however, the continuous use of a face mask can create adequate conditions for the proliferation and storage of pathobiont or opportunistic bacterial species that harm health; therefore, the constant replacement or washing of face masks is recommended as a preventive measure.

Financing

This study was financed with our own resources.

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