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Original Research Paper

Infectious risks of repeated and prolonged wearing of surgical masks during Covid-19

Surgical masks and Covid-19. Utility

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Abstract

Many countries have imposed the wearing of masks in public places in order to limit and reduce the rate of contamination as well as the transmission of the SARS CoV-2 virus. Unfortunately, many people have disregarded the safety precautions associated with using surgical masks appropriately, such as wearing the same mask for extended periods of time, hence the risk of infection with serious consequences. The goal of the current study was to assess this risk in this particular setting. Students at University Center of Naâma were given a questionnaire in order to gather data about this. As a consequence of the survey, it was found that every student had worn their surgical masks irregularly for more than two weeks. Analysis of microbiological data showed that both Gram-positive and Gram-negative bacteria were involved, mainly *Staphylococcus sp*. and *Pseudomonas sp*. as well as fungal species including *Candida albicans*, *Aspergillus sp.* and *Penicillium sp*. It should be noted that all of these microorganisms were able to form biofilms. In addition, their sensitivity to a range of families of antibiotics has varied; still, gentamicin shown efficacy against every strains examined in this investigation. As conclusion, failure to comply with health instructions regarding the preventive use of surgical masks could result in a reciprocal risk of infection.

Keywords: Covid-19, surgical masks, biofilm, resistance, infectious risk.

الملخص

من أجل تقييد وتقليل معدل التلوث وكذلك انتشار فيروس السارس، جعلت العديد من الدول ارتداء الأقنعة إلزاميًا في الأماكن العامة. للأسف، تجاهل العديد من الأشخاص احتياطات السلامة المرتبطة باستخدام الأقنعة بشكل مناسب، مثل ارتداء نفس القناع لفترات طويلة من الزمن. من هنا خطر الإصابة بالعدوى مع عواقب وخيمة، ولذلك كان تقييم هذا الخطر في هذا السياق المحدد هو هدف هذه الدراسة. من اجل جمع البيانات، تم توزيع استبيان على طلاب المركز الجامعي النعامة. وفقًا لنتائج الاستطلاع، تبين أن جميع الطلاب ارتدوا أقنعتهم بشكل غير منتظم لأكثر من أسبوعين. كشف تحليل البيانات الميكروبيولوجية عن تورط كل من البكتيريا إيجابية الجرام وسالبة الجرام في المقام الأول، وكذلك المكورات العنقودية والزائفة، إلى جانب الأنواع الفطرية مثل الرشاشيات، والمبيضات البيضاء، والبنسيليوم. وتجدر الإشارة إلى أن كل هذه الكائنات الحية الدقيقة قادرة على تكوين الأغشية الحيوية. بالإضافة إلى ذلك، تباينت حساسيتها لمجموعة من عائلات المضادات الحيوية؛ ومع ذلك، أظهر الجنتاميسين فعاليته ضد كل السلالات التي تم فحصها في هذا البحث. في الختام، فإن عدم الالتزام بالتعليمات الصحية المتعلقة بالاستخدام الوقائي للأقنعة الجراحية يمكن أن يؤدي إلى خطر متبادل للإصابة بالعدوى.

الكلمات المفتاحية: كوفيد-19، الأقنعة الجراحية، الغشاء الحيوي، المقاومة، مخاطر العدوى.

Introduction

Humanity has experienced numerous pandemics over time, SARS-CoV-2 has radically imposed new behaviors. This spherical virus (60 - 220 nm), surrounded by a helical protein capsid, is constantly developing following genetic mutations leading to new variants (Velavan and Meyer, 2020; Wu et al., 2020; Derouiche et al., 2023) which spread rapidly and cause numerous disabilities and deaths (Gorbalenya et al., 2020; Tadj and Seddiki, 2021). SARS-CoV-2 is mainly transmitted by air following contact with an infected person. Indeed, droplets emitted following coughs or during discussions can be inhaled by people nearby (1 à 2 m) (Van Doremalen et al., 2020). Indirect transmission is also possible, this is the emission of aerosols when respiratory droplets disperse in the air, these are capable of spreading over a large distance and causing infection (Adhikari et al., 2020; Umakanthan et al., 2020).

To limit the circulation and transmission of the virus between people, the World Health Organization (WHO, 2009) recommended the use of masks in public places to prevent the inhalation of SARS-CoV-2 particles and other respiratory viruses. For this, countries around the world have declared the obligation to wear masks in these places as a means of protection. According to the NF EN 149 standard (2009), single-use respiratory protection masks protect the individual against the inhalation of droplets or particles suspended in the air. However, a surgical mask is a medical device (EN 14683 standard) which prevents the projection of droplets and limits contagion. According to the WHO (2020), these masks should under no circumstances be used beyond 4 hours.

A surgical mask is made up of three sheets superimposed one on top of the other. The inner one is made of hydrophilic cotton fibers to absorb moisture from the nasal and oral orifices. The intermediate sheet is made of polypropylene, a non-woven fiber with thermoplastic and hydrophobic properties. This sheet is used as a filtration barrier (Donovan and Skotnicki-Grant, 2007). The first two sheets are covered by a non-absorbent polyester outer layer, which prevents the penetration of droplets (Corradini et al., 1967).

On the other hand, many microorganisms are capable of adhering to different surfaces and installing complex structures called biofilms (Seddiki et al., 2015). These structures are microbial communities organized and adhered to biotic or abiotic surfaces and are characterized by the secretion of a polymer matrix (Costerton et al., 1978); it must be emphasized that the colonization and adhesion of micro-organisms becomes important on a rough support (Characklis, 1990). With this in mind, William Costerton (1999) highlighted the link between the risk of infection and the formation of biofilms. The dangerousness of the biofilm is linked to the bacterial and/or fungal species which form these complex structures (Jaballah et al., 2006; Seddiki et al., 2015). Resistance is one of the properties that characterize microbial biofilms (Hawser and Douglas, 1995; Ramage et al., 2012). Within this framework, the current study sought to assess the infectious risk associated to the use of the contaminated surgical masks. For this reason, a survey about students' use of masks during the Covid 19 pandemic was conducted at the Nâama University Center.

Equipment and methods

Sampling

The sample concerned the masks used by students of the Naâma university center during their presence in the center. The target population includes a mosaic of students, girls and boys, belonging to the various institutes of the establishment, after having obtained their agreements.

The samples were taken in February and March 2022. The used masks were collected in sterile bags directly after removal. An assessment survey was carried out in parallel with the mask samples which was established according to the recommendations of the WHO (2020). Said survey is a questionnaire (Table 1) given to students who participated in the study, it brings together the personal data of participants (age, sex, etc.) and those relating to the practice of wearing masks, such as the frequency of use, whether or not masks are washed, their co-use with other people, where they are stored, etc.

For negative control tests, sterile surgical masks intended for surgeons in the operating room of Nâama hospital were used.

Table 1. Investigation data sheet.

Technical sheet

Reference : .... Student in ....

Gender : Men ☐ Female ☐ Age ....

Are you a regular user of the mask ? yes ☐ no ☐

If yes, frequency of use per day once several times specify ....

Do you wash/rinse your mask ? yes ☐ no ☐

Do you use other people's masks ? yes ☐ no ☐

Do you use the same mask outside the university center ? yes ☐ no ☐

If yes, what are the places often frequented ....

Where do you keep your mask for multiple uses ? ....

Were you a victim of SARSE CoV-2 ? yes ☐ no ☐

Do you suffer from allergies ? yes ☐ no ☐

If yes, treatment ....

Do you suffer from other illnesses ? yes ☐ no ☐

Please elaborate ....

Treatment ....

Do you smoke cigarettes ? yes ☐ no ☐

Where do you dispose of the mask after use ? ....

Microbiological count

Taking aseptic measurements, three fragments of 1 cm^2 were cut from the middle layer of the mask from the area adjacent to the oral cavity. These fragments were used for microbiological enumeration, enrichment and direct contact testing.

Two distinct techniques were used in parallel to count the microbiological particles attached to the mask fabrics. The first was an adaptation of the Brun-Buisson method (Brun-Buison et al., 1987) to samples of mask fragments; this protocol involves vortexing the sample for one minute to loosen microorganisms. The second was to apply the inner layer directly to the nutrient agar's surface for five minutes. This allows the growth and enumeration of microorganisms affecting the sample (Gund et al., 2021). The inocula were incubated at 37°C for 24 hours.

Sabouraud agar medium supplemented with 0.5 g/L chloramphenicol was used for fungal cells. The mask fragments were directly deposited on the agar and the plates were then incubated at 25°C for 7 days. For filamentous fungi, each strain was subcultured separately by taking a disc from the fungus then placing it in the center of a Petri dish containing Sabouraud agar (Botton and Breton, 1990).

Successive liquid/solid subcultures were carried out until pure strains were obtained which were then stored at 4°C.

Microbial identification

In order to highlight the different microbial forms, the fragments cut from divert masks were subjected to examination under an optical microscope.

ChromAgar, which is an orientation medium, has a great capacity for differentiation between species on the basis of their colors and morphologies (Stefaniuk, 2018). The isolated strains were inoculated on appropriate agar plates then incubated at 37°C for 24 hours.

According to the results of the choromogenic tests and, in order to obtain a precise identification, a series of biochemical tests was carried out using API 20E and Id 32 Staph identification galleries (Biomerieux, Lyon - France). These galleries constitute a miniaturized and standardized system of biochemical tests, they are designed for the respective identification of Enterobacteria and Gram-negative bacilli as well as *Staphylococcus*, *Micrococcus* and related genera, such as *Rothia sp*. and *Aerococcus sp*. The identity of the strains and the corresponding biotypes were revealed by referring to the identification table provided with the galleries.

The identification of yeasts was carried out according to the morphological appearance of the strains. Two culture examinations were carried out, the germination test in blood serum and the chlamydosporulation test (Taschdjian et al., 2018; Bouchet et al., 1989). The identification of filamentous fungi was based on the study of the macroscopic and microscopic characters of the colonies (Chabasse et al., 2009).

Formation of biofilms

The risk of infection increases significantly with the formation of biofilms (Peeters et al., 2008). Evaluation of this capacity in isolates is essential in order to determine this risk; however, the tissue culture plate method was used.

This quantitative technique, described by Christensen et al., (1982) then modified in 2000 by O'Toole et *al*. (2000), allows the evaluation of biofilms formed using 96-well microplates. Measuring absorbance using a microplate reader (OPTIC IVYMEN SYSTEM 2100C) reflects the quantity of the biofilm since it is proportional to the absorbance measured in each well of the microplates. The absorbance was measured at 630 nm for bacteria and 492 nm for yeast. Each test was carried out in triplicate for each strain.

Absorbance values were presented as mean ± standard error of the means. Table 2 indicates the classes of strains according to their biofilm formation potential, these have been listed in four classes (Stepanović et al., 2007).

Table 2. Classification of biofilm formation potential in microorganisms (Stepanović et al., 2007).

Absorbance	Formation of biofilm
A ≤ At	**-**
At < A ≤ 2 × At	**±**
2 × At < A ≤ 4 × At	**+**
A > 4 × At	**++**

At: Control absorbance of negative controls. -: Absence, ± : Weak, + : Moderate, ++ : Strong.

Statistical analyzes

The results of the study were analyzed using R-studio software. A multiple correspondence analysis (MCA) as well as a statistical description of the results were carried out.

Descriptive statistics were used to classify the microorganisms present in surgical masks and to assess the potential of biofilm formation. Multiple correspondence analysis was used to illustrate confidence ellipses around the modalities of all the variables studied.

Results and discussion

Sampling

According to the survey carried out, the age range of the participants varies between 19 and 38 years old. All students who participated in the survey had used their surgical masks irregularly, 70% of them used these masks inside the university center as well as outside, particularly public places. Some students are part of the medical staff at Naâma hospital, they surprisingly used the same masks in the hospital and at the university center. Students seem to be at high risk from this attitude.

In addition, the frequency of use of surgical masks varies. In fact, 53.85% of the targeted population wore the same masks for a week, while 46.15% of them used the same masks beyond two weeks. Although 7.69% of students used chemicals to clean their masks, such as bleach and soap, most students used their masks for a long time without any washing. Curiously, according to the same survey, 69.23% of the students included in this study indicated that they had thrown away their masks in inappropriate places after removing them.

Although more than half of the students were not vaccinated against SARS CoV-2 during the survey, the majority of participants (61.54%) experienced allergic attacks, shortness of breath and dental inflammation during the wearing of masks. As a result, the confidence circles obtained in the multiple correspondence analysis (MCA) plotellips illustrated confidence ellipses around the modalities of all the variables studied (Figure 2), the male category of the age group from 30 to 38 years old (named adult in the plotellips) have been victims of SARS-Cov-2 more than those aged 19 to 29 (called adolescents), or even the woman category. The MCA revealed a link between the very frequent use of masks and the development of an allergy to them.

Analysis of the questionnaire data revealed that the majority of participants did not respect the health protocol relating to the proper use of surgical masks. Thus, irregular use of masks may be one of the reasons responsible for the risk of infection; this study consolidates this observation.

Contrary to our results, Monalisa et al., (2017) found in a private dental university that 16% of students wore the same masks throughout the day. However, the inappropriate locations where masks were discarded were similar to those in the present study (Monalisa et al., 2017). Previous studies (Luksamijarulkul et al., 2014; Zhiqing et al., 2018) mentioned that the use of surgical masks creates warm and humid microclimates, which favors the accumulation and growth of microorganisms, hence the risk of infection.

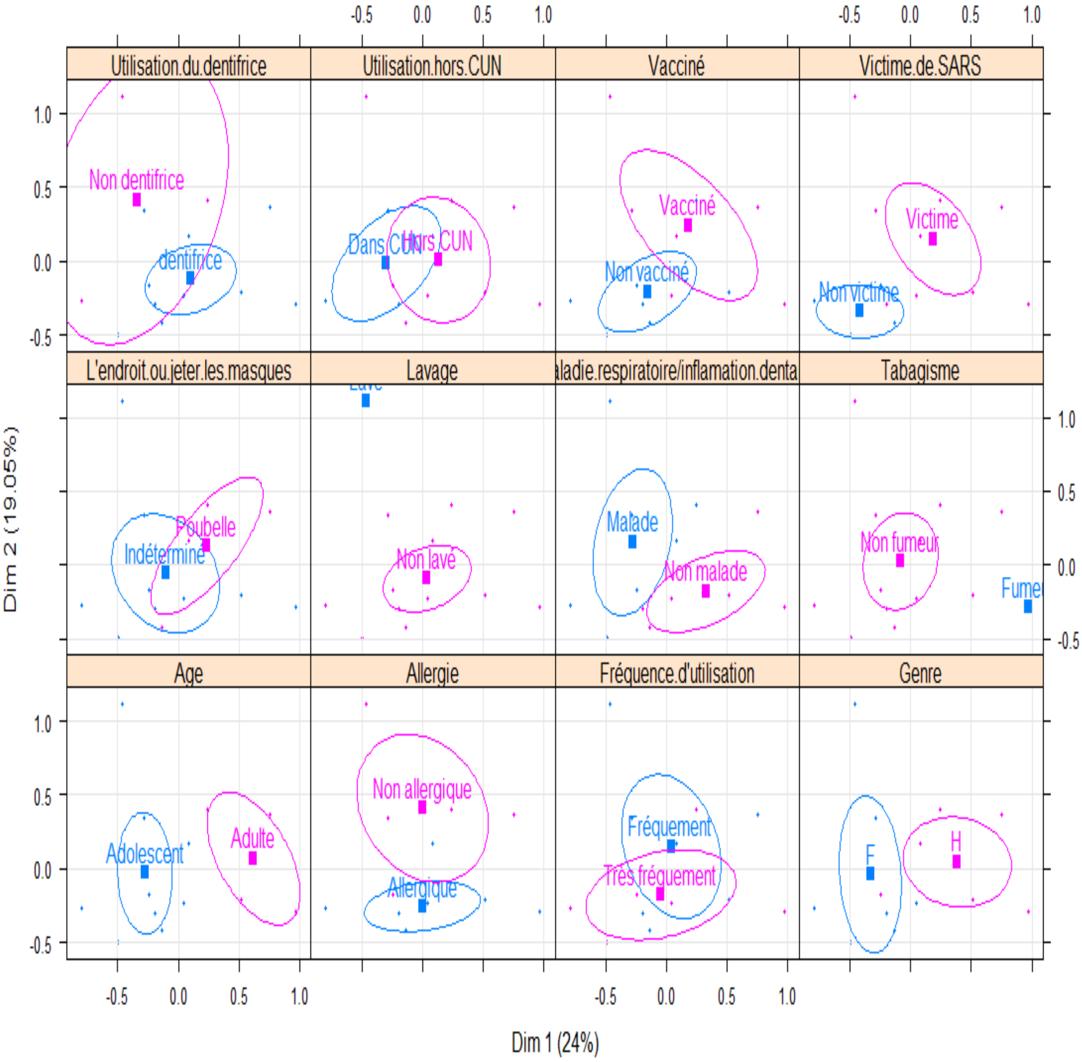

Figure 2. Multiple correspondence analysis plotellips illustrating confidence ellipses around the modalities of all the variables studied.

Microbiological enumeration

The counting test revealed the contamination of surgical masks used by the student population. An average of five colony-forming units per square centimeter of the middle layer of these masks was revealed (5±0,3 UFC/cm^2). In fact, the polypropylene nature of the middle layer of surgical masks is one of the factors that promotes the covalent adhesion of microorganisms (Tadielo et al., 2022). In addition, shaking the sample for one minute does not allow all the sessile cells that form biofilms to detach (Boucherit-Atmani et al., 2011**).** This partly explains the reduced number of colonies formed on nutrient agar by the technique of Brun-Buisson et al., (1987). Despite the medical relevance of these results, a number lower than 5 CFU cannot be taken into consideration for the assessment of the microbial load (Breed and Dotterrer, 1916).

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Unlike this first protocol, that of Gund et al., (2021) allowed significant microbial growth ($P<0,05$); the number of colonies formed on nutrient agar turned out to be incalculable.
In addition to their high number, different shapes and sizes of microbial colonies were observed. This result demonstrates the diversity of microorganisms retained by the surgical mask.

Microbial identification

By examining the internal part of the masks under the optical microscope (Figure 3), pores with diameters of approximately 0.086±0.004 mm were observed. The ultra-architectural aspect of this part suggests that the microorganisms were able to pass through these pores and therefore regain the intermediate layer of the masks. These masks, made of polypropylene, seem to prevent the inhalation of droplets and aerosols, hence the adhesion of microorganisms (Donovan and Skotnicki-Grant, 2007).

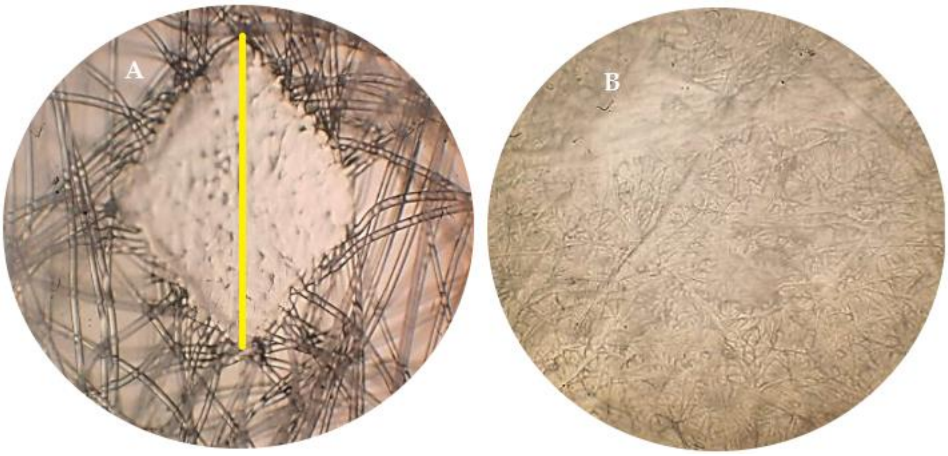

Figure 3. Microscopic observation of a surgical mask of the inner (A) and intermediate (B) layers. The yellow bar designates the diameter of the pore. (Magnifications × 1000).

Since the internal parts of surgical masks come into contact with certain parts of the wearer's face, the areas around the nose as well as the inner corner of the eyes are the most contaminated compared to other areas of the face (Nejatidanesh et al., 2013). Other results showed that nasopharyngeal pathogens excessively accumulate outside and inside the mask when breathing (Kappstein, 2020). This requires particular attention when using this type of mask long term.

It should be noted that the study carried out by Gund et al., (2021) revealed that all masks used by student dentists during their practice had microbial contamination in their internal layers; This finding confirms the importance of this layer.

On the other hand, it emerges from the microbial isolation (Figure 4) a clear dominance of bacterial strains (68%). Filamentous fungi ranked second with an isolation rate of 26%. Yeast cells were fewer; only three strains were isolated.
According to a previous study, masks cause moisture retention, creating a warm and humid microclimate conducive for ideal growth of various microbial agents, such as bacteria and fungi (Jamjoom et al., 2009).

Based on their macro-chromogenic and biochemical aspects, the identity of the isolated bacterial strains revealed six species; these are *Staphylococcus aureus*, *Staphylococcus epidermidis*,

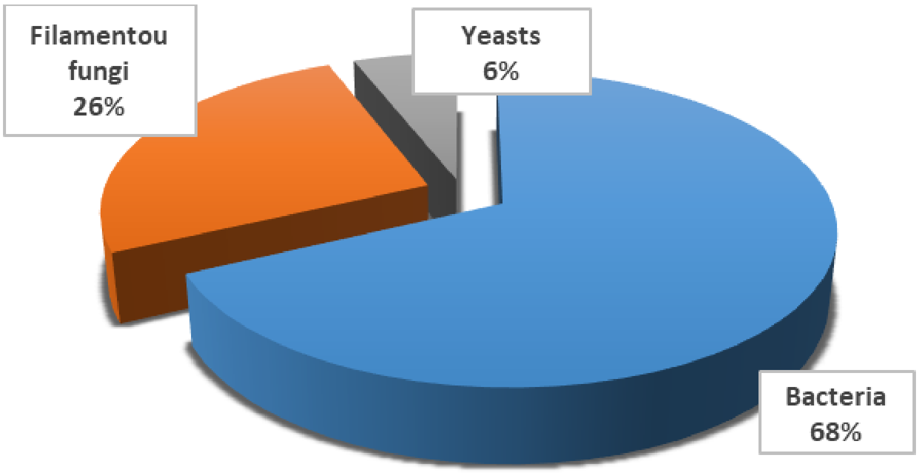

Figure 4. Distribution of microbial strains isolated from used surgical masks

Pseudomonas aeruginosa, *Pseudomonas fluorescens*, *Proteus vulgaris* and *Serratia plymuthica*.

Consistent with the present results, other studies reported the diversity of pathogens contaminating surgical masks and specified that they were *Staphylococcus sp*., *Pseudomonas sp*. and also *Candida* yeasts **(**Luksamijarulkul et al., 2014; Zhiqing et al., 2018).

Furthermore, the presence of filamentous fungi seems to indicate airborne contamination. Microscopic examinations revealed different aspects of filamentous fungi (Figure 5) dominated by *Aspergillus sp*. (34%), and then *Penicillium sp*. and *Mucor sp*. (25%). In contrast, *Curvularia sp*. and *Alternaria sp*. were isolated with a low rate, estimated at 8%.

The filamentous fungi involved in the pathologies are indeed diverse, many of them are responsible for chronic colonization of the respiratory tract (Chabasse et al., 2009).

Luksamijarulkul et al., (2014) documented in this context the importance of contamination of surgical masks by *Aspergillus sp*. and *Penicillum sp*. used by medical personnel. Other authors have demonstrated that *Aspergillus sp*. causes secondary infections in patients with Covid 19; which corroborates the results of this study (Bassetti et al., 2020; Lai et al., 2021, Marr et al., 2021). In fact, two-thirds (2/3) of the students included in the survey suffered from Covid 19, while more than half suffered from allergies and shortness of breath.

Of interest, *Penicillium sp*. can cause clinical manifestations on human health, it is considered a very allergenic fungus (Reboux et al., 2019). On the other hand, *Mucor*, also isolated from surgical masks, is responsible for mucormycosis, this rare infection can still affect the lungs and skin of patients (Lakhdar et al., 2016). Additionally, *Alternaria sp*. is considered an aeroallergenic fungus, it is involved in the occurrence of asthma and allergic rhinitis (Fung et al., 2000; Thibaudon et Lachasse, 2006). However, *Curvularia sp*. is considered responsible for skin infections (Gunathilake et al., 2014).

Furthermore, microscopic and biochemical examinations of microbial cultures revealed three strains of *Candida albicans*. This species is responsible for numerous oral and dental pathologies

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(Malbos, 2022). Its isolation from surgical masks therefore appears logical.

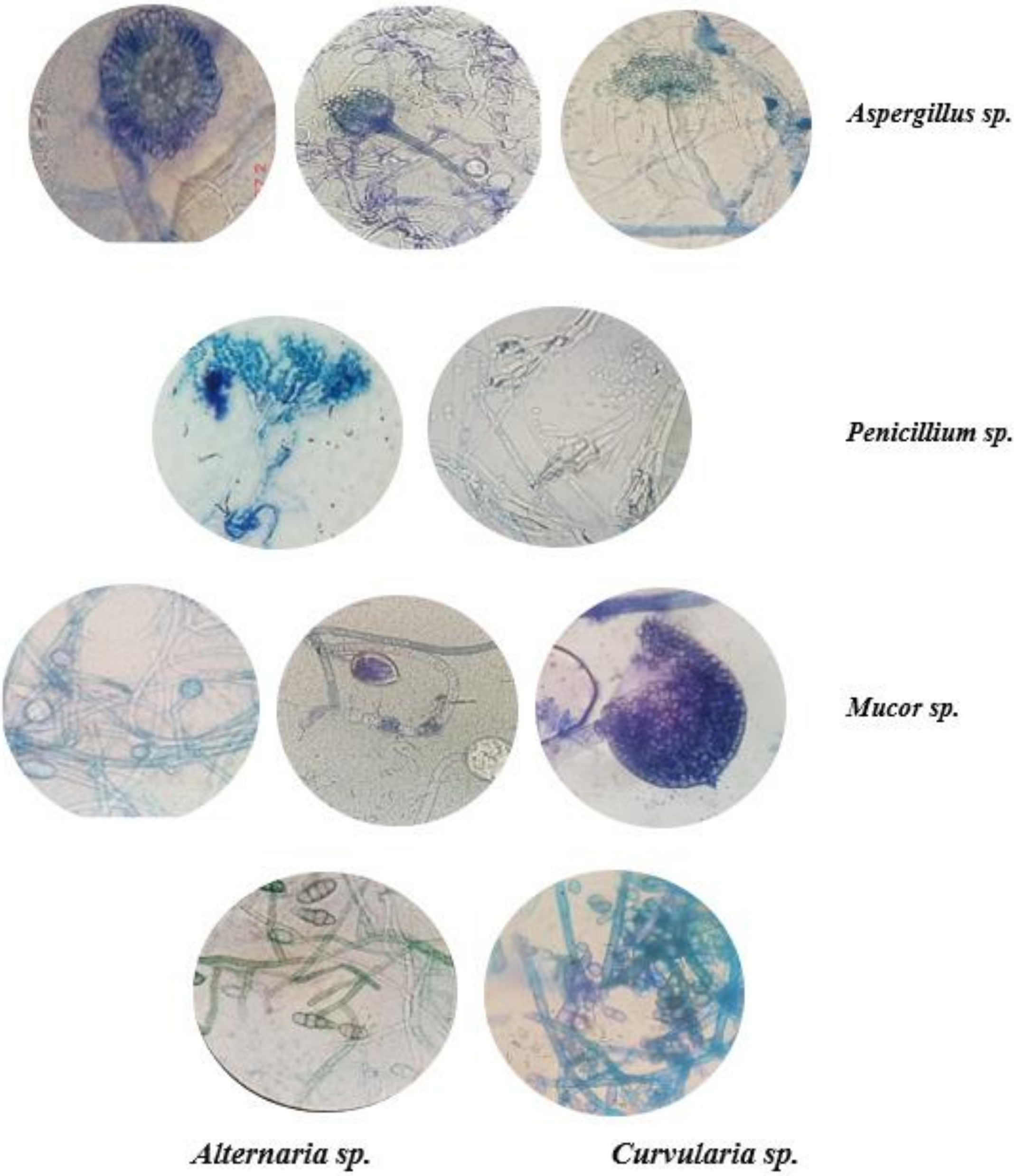

Figure 5. Microscopic aspects of filamentous fungi isolated from masks.

Formation of biofilms

The method used to evaluate the potential for biofilm formation revealed this power in all bacterial strains. Indeed, the microplate method continues to have a special position in the screening of biofilm formation *In Vitro* (Lozano et al., 2022).

According to the data, 29.41% of the strains had a high potential for biofilm formation, and 55.88% of the strains had a moderate potential. However, only 14.7% of strains were classified as weakly biofilmogenic. Recent findings by Tadielo et al., (2022) reveal that Gram-positive and Gram-negative bacilli live in communities on polypropylene surfaces, which is the material used to make the inner layer of surgical masks. This could help to partially explain why these species produce biofilms.

One infectious risk factor is the possibility of biofilm development (Costerton, 1999). This capacity is an intrinsic character in many bacterial species but can evolve within the same species depending on its environment (Donlan and Costerton, 2002; Seddiki, 2021).

However, the findings of this investigation demonstrated that certain strains of *Candida albicans* are effective biofilm formers. In fact, this species grows a biofilm on polypropylene materials (Wang et al., 2021). Given that *Candida sp*. is the tenth potentially pathogenic microorganisms for humans (Baghad et al., 2021), using contaminated surgical masks for an extended period of time increases the risk of infection. It should be mentioned that on polypropylene surfaces, sessile bacterial cells coexist harmoniously inside the same biofilm (Tadielo et al., 2022).

Conclusion

The repeated and prolonged use of the same surgical masks is the subject of this study, which aims to be pioneering in Algeria. This risk primarily concerns people who use these devices but do not follow the health instructions relating to their proper use, particularly during pandemics such as Covid-19.

The investigation carried out at the Nâama university center found that students, on the whole, did not respect health instructions. Most of them experienced allergies related to wearing surgical masks, especially if they did so for longer than a week. In addition to having an adverse effect on the users, this careless use may also have unintended consequences for the ecosystem. In actuality, most of the participants threw these masks in inappropriate places.

Through this study, we were able to enumerate, isolate, identify and evaluate the potential for biofilm formation. Overall, the inner layers of surgical masks, included in this study, were contaminated with a vast array of microorganisms. However, when the polypropylene intermediate layer was evaluated using the Brun-Buisson approach, the actual amount of contaminated microorganisms was not disclosed. While certain fungal species, including *Aspergillus sp., Penicillium sp.,* and *Candida albicans*, have been identified from surgical masks, the majority of the samples were bacteria. These are typically *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Serratia plymuthica* and *Proteus vulgaris*. These results lead to great vigilance when using surgical masks in order to prevent possible infectious risks.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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