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Do face masks increase the rate of the *Staphylococcus aureus* nasal carriers?

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Sir,

Staphylococcus aureus is a Gram-positive bacteria relevant in clinical practice. This microorganism can be part of the skin and mucosal microbiota, but it can also cause diverse infections.

When colonizing *S. aureus* presents its main reservoir in the nasal mucosa, being this location one of the best to evaluate *S. aureus* carriage. There has also been described a correlation between nasal carriage and the possibility of suffering an infection [1]. The prevalence of colonization in healthy population is very high. Longitudinal studies suggest that 20% of the population are persistent carriers, 30% of the population are intermittent carriers and 50% are not colonized [2].

In a study performed at the Complutense University of Madrid in pre-clinical Podiatry students over two academic years, between 2018-19 and 2019-20, a prevalence of 22.7% of *S. aureus* nasal carriers was observed before the pandemic and the widespread use of face masks [3].

Several studies have shown the efficacy of face masks in preventing the transmission of viral diseases [4], as well as in preventing bacterial diseases such as meningitis [5]. However, as far as we know no studies have been found in the literature that analyse the impact of continuous use of face masks on the pandemic in the *S. aureus* carriage.

The objective of this study was to compare the evolution of *S. aureus* nasal carriage in a population of pre-clinical students of Podiatry and Medicine in the COVID-19 pandemic, in the 2021-22 academic year with a face mask, and in the academic year 2022-23 without a face mask.

A descriptive and transversal study was conducted in the Microbiology Area of the School of Medicine with 162 volun-

tary students of the Complutense University of Madrid. Out of these, 37 and 58 students coursed Podiatry in the academic year 2021-22 and 2022-23, respectively; whilst 31 and 36 coursed Medicine in the academic year 2021-22 and 2022-23, respectively.

Since the return of face-to-face classes in the academic year 2021-22, the use of face masks was mandatory in Spain due to the COVID-19 pandemic and, the use of face masks indoors ceased to be mandatory on 22nd April 2022. Therefore, in the academic year 2022-23 the use of face masks was no longer mandatory. In the Figure 1, we can observe the chronological sequence of when the samples were taken compared to each modification of COVID-19 pandemic regulation.

The samples were collected during the practical classes of Microbiology. Each swab was rubbed against the anterior of the nasal vestibular wall of both nares and immediately placed in 10 mL of Mueller-Hilton broth (BD™ Difco™) at 7,5% NaCl concentration and was then incubated at 35°C for 24 hours. Afterwards, swabs were vortexed for 20 s before plating onto Mannitol salt agar (BD™ Difco™), incubated at 35°C for 24 hours. The isolates were characterized as *S. aureus* based on morphology, Gram stain, catalase test, coagulase test, and mannitol salt agar fermentation. Once *S. aureus* was identified, methicillin susceptibility was determined by disk diffusion.

A Fisher's test was performed to compare the different proportions of *S. aureus* carriers during the academic years of the podiatry degree and the medical degree separately. For the joint analysis, a Chi-square test was performed to compare the different proportions of *S. aureus* carriers during the academic years of the Podiatry degree and the Medicine degree.

In Figure 2 we can observe the evolution of prevalence in *S. aureus* nasal carriage in students of Podiatry and Medicine in both academic years. In Podiatry degree, 11 students (29.7%) and 11 students (19.0%) were *S. aureus* nasal carriers in the academic years 2021-22 y 2022-23, respectively. Regarding Medicine degree, 10 students (32.3%) y 8 students

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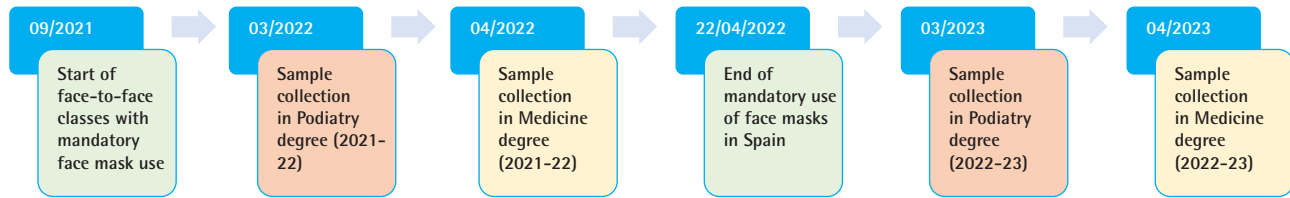


Figure 1 | Flow chart study

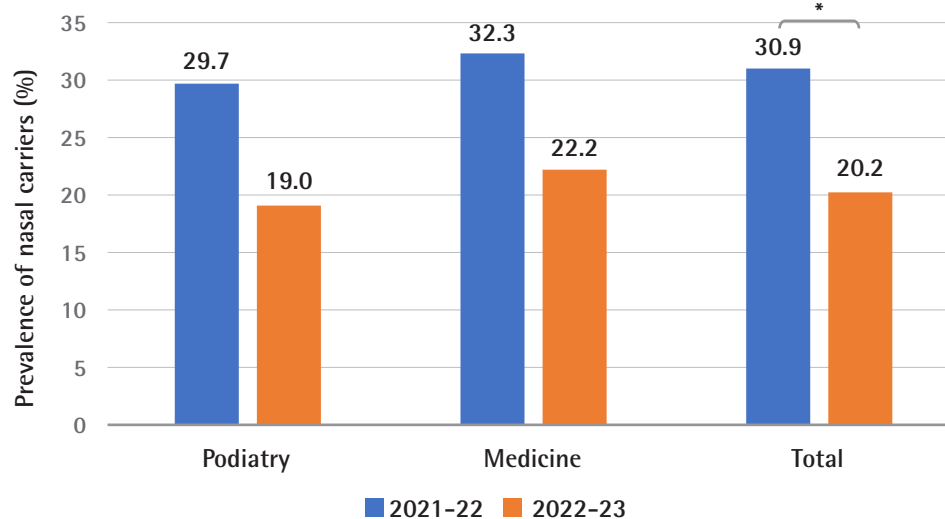


Figure 2 | Prevalence of nasal carriers in the pre-clinical students of Podiatry and Medicine in the studied academic years.

* $p=0.0004$

(22.2%) were *S. aureus* nasal carriers in the academic years 2021-22 y 2022-23, respectively. In both cases the percentage of *S. aureus* nasal carriers were higher in the year 2021-22 compared to the year 2022-23, however the differences were not statistically significant ($p=0.22$ and $p=0.35$). Nevertheless, when all students were analyzed jointly, the percentage of *S. aureus* nasal carrier was statistically higher in the year 2021-22 (30.9%) compared to the year 2022-23 (20.2%) ($p=0.0004$). In none of the academic years, a methicillin-resistant *S. aureus* was isolated.

The results of the study show a higher prevalence of *S. aureus* nasal carriers in both Podiatry and Medicine students during the mandatory use of face masks in the 2021-22 academic year compared to the 2022-23 academic year. These differences were not statistically significant, probably due to the sample size. However, when analysing the students as a whole, the percentage of *S. aureus* carriers was significantly higher in the 2021-22 academic year compared to the 2022-23 academic year.

If we compare our data with the prevalence percentages of *S. aureus* from previous years, we can see that the data for the 2022-23 academic year are similar to those obtained with students of the Podiatry degree in the 2018-19 and 2019-20 academic years, where a percentage of carriers of 21.4% and 23.6%, respectively, was observed [3].

The use of face masks is known to produce a number of effects, mainly nasal discomfort, breathing difficulties, excessive sweating, and pain around the ears [6,7]. Nasal discomfort may be attributable to the warm air and humidity present under the mask. Wearing a face mask may increase the oral temperature in healthy subjects [8]. Thus, one possible explanation for the increased prevalence of *S. aureus* carriers is that it may be related to the increased temperature and humidity in the nostrils caused by the face masks. Although this bacterium is able to grow between 6°C and 40°C, its optimal growth temperature is between 30°C and 37°C [9].

It has also been observed that factors such as viral mi-

crobiota are able to directly influence the bacterial microbiota [10] Thus, mild viral nasopharyngeal infections lead to an enrichment of aero-tolerant bacteria, including *S. aureus* [10]. The presence of SARS-CoV-2 during the pandemic may also have produced a variation in the nasal microbiota.

It is important to note that this study was conducted in pre-clinical students, as contact with the hospital environment has been observed to be a risk factor for nasal colonisation by *S. aureus*. Thus, a cohort study analysing a class of medical students at the Complutense University of Madrid during their in-hospital training between the third and sixth year at the Hospital Clínico San Carlos showed a statistically significant increase in the prevalence of nasal *S. aureus* carriage, which was 26.9% in students who had not had hospital training and 46.2% in students in the sixth year [11].

The main limitation of the study is that it is not a longitudinal study as the students were not followed up. We can conclude that in a preclinical student population of Podiatry and Medicine students, the use of face masks was correlated with an increased prevalence of *S. aureus* nasal carriers. Our results confirm that active carrier detection of *S. aureus* among future healthcare professionals is necessary to break the possible chain of transmission in different healthcare settings.

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None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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