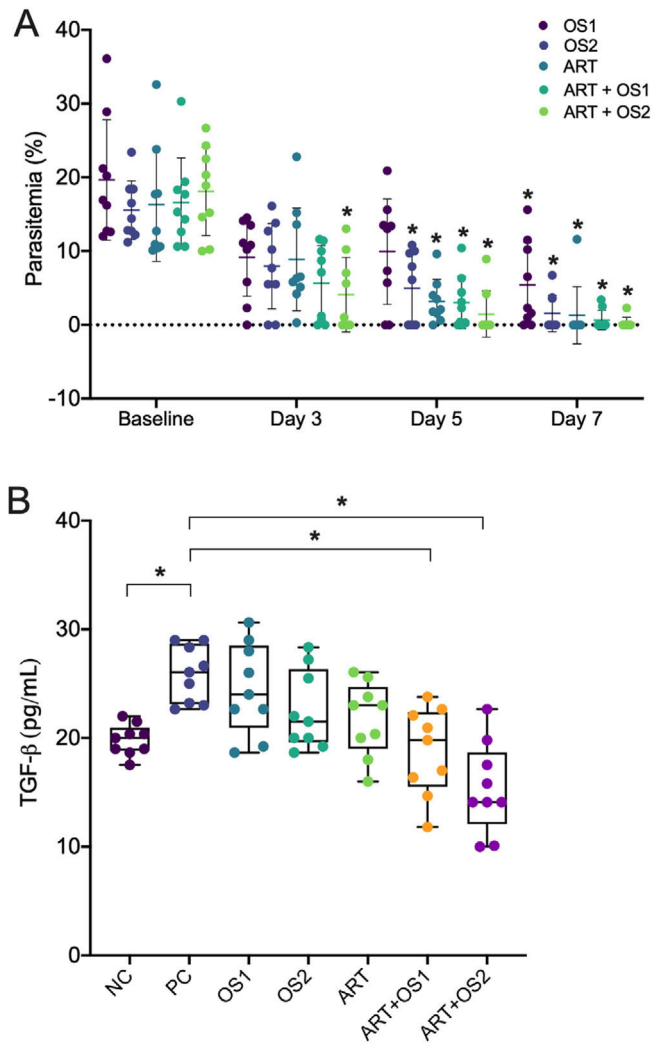

Fig. 1. Synergistic anti-malarial effects of *Ocimum sanctum* leaf extract and artemisinin. (A) Reduction of parasitemia. (B) TGF-β level by peritoneal macrophages during mice malaria infections measured by ELISA after 24 h *in vitro* culture. Data are expressed as a mean ± standard deviation (SD). (*) Indicates a significant difference compared to the baseline level or between two groups indicated in the graphs ($p < 0.05$). OS1 = *O. Sanctum* 0.25 mg/g/day; OS2 = *O. Sanctum* 0.5 mg/g/day; ART = artemisinin 0.036 mg/g/day; ART + OS1 = artemisinin 0.036 mg/g/day + *O. Sanctum* 0.25 mg/g/day; ART + OS2 = artemisinin 0.036 mg/g/day + *O. Sanctum* 0.5 mg/g/day; NC = negative control; PC, positive control.

Conflict of interest

None to declare.

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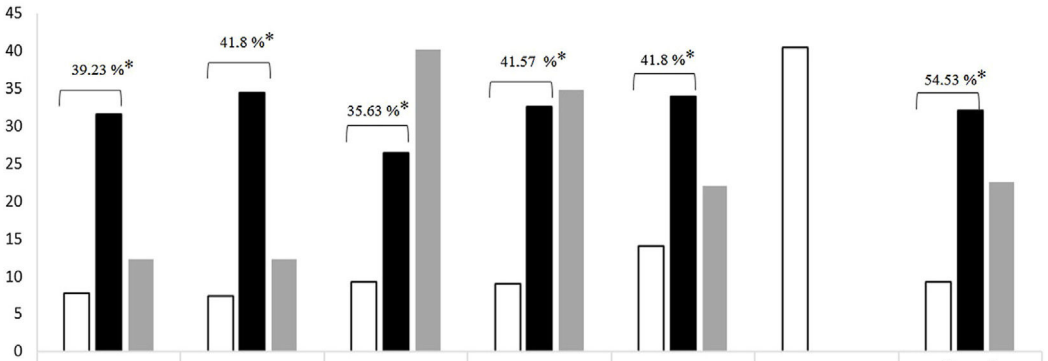
High rates of colonization by *Staphylococcus aureus* in medical students before their clinical practices

Elevadas tasas de colonización por Staphylococcus aureus *en los estudiantes de medicina antes de realizar sus prácticas clínicas*

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a major public health problem. Active carrier detection is recommended among healthcare staff when there are nosocomial outbreaks or in highly endemic situations in certain risk areas.[1] As future healthcare professionals, medical students must be aware of their role as potential sources of *S. aureus* transmission to patients. Our study had the following objectives: (a) to determine the carrier rate (nasal and/or pharyngeal) of methicillin-sensitive *S. aureus* (MSSA) and MRSA among third-year medical students at Universidad Complutense de Madrid, in Madrid, Spain, who had not yet started their practical placements at Hospital Clínico San Carlos; and (b) analyse possible risk factors.

Over six consecutive academic years (from 2014 to 2022), all students voluntarily had a pharyngeal and nasal sample taken, except in the 2021/2022 academic year when, due to the SARS-CoV-2 prevention and safety measures, the student's themselves



	2014-15	2015-16	2016-17	2017-18	2018-19	2021-22	Overall (2014-2019)
Nasal (%)	7.69	7.38	9.20	8.99	14	40.51	9.3
Nasal and Pharyngeal (%)	31.54	34.43	26.44	32.58	34		32
Pharyngeal (%)	12.31	12.30	40.23	34.83	22		22.54

Figure 1. Distribution of the rates of *Staphylococcus aureus* carriers according to the academic year and location (nasal, pharyngeal and both).
*Total percentage of students colonized at least in the nose.

took a nasal sample only. Inoculation was immediately carried out in two culture media for the isolation and identification of *S. aureus*: chromID SAID agar (bioMérieux *S. aureus*); and chromID MRSA agar (bioMérieux *S. aureus*). The students also anonymously completed a questionnaire assessing possible factors associated with a carrier state, such as gender, chronic sinusitis, acute sinusitis, taking antibiotics in the 10 days prior to the sample, hospital admissions or interventions and related local surgery. Chronic sinusitis was defined as inflammation of the sinuses with signs and symptoms lasting for at least 12 weeks.

The statistical analysis of the results was carried out with the Epi Info 7.2.5.0 program, considering a statistically significant result if $p < 0.05$.

Of 607 students included, 369 (60.8%) were colonized by *S. aureus*, nine of them (1.5%) being carriers of MRSA. This overall colonization rate groups all types (nasal, pharyngeal and both), which is why it is so high compared to other studies that only take into account the culture of a nasal sample to assess *S. aureus* carrier status.[2–5] However, it has been reported that if the pharyngeal culture is added to the nasal culture, detection sensitivity can be increased by 25.7%.[6]

The prevalence of MRSA found in the general population and pre-clinical students is in the range of 0.8%–1.8%.[7,8] We obtained an MRSA colonization rate of 1.5%, within these margins.

Fig. 1 shows the distribution of colonized students according to the academic year and the location (nasal, pharyngeal or both). A total of 250 students (41.2%) were colonized at least in the nose, this rate being higher than that described in some university students in our area.[2,3] However, other studies carried out in pre-clinical students report colonization rates of 40.8%[4] and 43.6%.[5] Where *S. aureus* colonizes the nose, with only a few exceptions, the bacterium is also present in the pharynx.[9] In our study, 77.52% (169/218) of the students with nasal isolation of *S. aureus* were also colonized in the pharynx, and this association was statistically significant ($p < 0.001$).

Although the primary *S. aureus* colonization site is the nostrils, some studies have isolated *S. aureus* more frequently from the pharynx than from the nose.[9] Our results show that exclusively pharyngeal colonization was always more common than exclusively nasal colonization (22.53% vs 9.3%, $p < 0.001$).

With regard to *S. aureus* carrier status, people with chronic sinusitis have been found to be at greater risk of being carriers.[10] We found statistically significant differences between students colonized and non-colonized with *S. aureus* in the nose regarding chronic sinusitis (19.2% vs 8.4%, $p < 0.001$). These data are consistent with the results from a previous study we conducted in 2008 on pre-clinical students from the same hospital.[2]

In short, our study shows a high rate of *S. aureus* colonization among third-year medical students, future healthcare professionals, with exclusively pharyngeal colonization being more common than that in the nose.

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Conflicts of interest

None.

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Intermittent bladder irrigation with liposomal amphotericin B for the treatment of fluconazole-resistant *Meyerozyma guilliermondii* cystitis in an immunosuppressed adolescent

Instilaciones vesicales intermitentes con anfotericina B liposomal para el tratamiento de una cistitis por Meyerozyma guilliermondii *resistente a fluconazol en un adolescente inmunodeprimido*

Urinary tract infection (UTI) is the third leading cause of nosocomial infection in paediatrics.[1] Although most infections are of bacterial origin, fungal aetiology is increasing due to *Candida* species with less sensitivity to azoles.[2] The treatment of these infections is complex due to a lack of therapeutic options.

We present the case of a 16-year-old patient with a left pelvic metastatic osteosarcoma undergoing chemotherapy who required permanent bladder catheterisation because of tumour compression. He had a history of urinary infections in the previous two months caused by *Candida parapsilosis* and *Meyerozyma guilliermondii* (*Candida guilliermondii*), which had been resolved by replacing the catheter and treatment with fluconazole. After receiving a cycle of chemotherapy and being in a state of neutropenia, the patient developed sepsis of abdominal origin, requiring broad-spectrum antibiotic therapy with meropenem, amikacin and vancomycin. In this context, *M. guilliermondii* was isolated in his urine, persisting in successive urine cultures despite the catheter being replaced once again and treatment with intravenous fluconazole for two weeks. Blood cultures were negative and renal ultrasound showed no significant findings apart from bilateral pyelocalyceal dilation secondary to obstruction caused by the tumour mass. The antifungal susceptibility tests showed resistance to fluconazole (MIC 16 μg/mL), with sensitivity to the other antifungals tested. With these findings, it was decided to start combined treatment with intravenous micafungin, which was continued for 14 days, and daily bladder irrigation with liposomal amphotericin B (30 mg/100 ml); 100 ml was introduced through the catheter, clamped for 10 min only. The treatment was well tolerated, with no adverse effects detected, and it was continued for five days, achieving microbiological eradication.

Treating asymptomatic candiduria is not indicated in paediatrics unless the patient develops symptoms or belongs to a group at risk of spread (neutropenia, need for urological manipulation, newborn or a kidney transplant). The treatment of choice is oral fluconazole,[3,4] with amphotericin B deoxycholate, flucytosine, or bladder irrigation with amphotericin B deoxycholate recommended for resistant species.[3] In our case, amphotericin B deoxycholate is not available for use in Spain, and flucytosine was ruled out because it is associated with myelotoxicity in up to 22% of patients.[5] Liposomal amphotericin B, triazole derivatives and echinocandins have limited excretion in the urine. We opted for intermittent bladder irrigation with liposomal amphotericin B, in combination with micafungin as systemic treatment in view of the patient's neutropenia. The concentration of echinocandins in urine is very low (0.7% of the plasma concentration in the case of mucafungin). Still, there are reported cases of successful use in the treatment of candiduria due to *Candida* species resistant to fluconazole,[6,7] although there have also been therapeutic failures.[8] We cannot, therefore, rule out that micafungin may have contributed to our patient being cured.

Most of the literature consulted for bladder irrigation with amphotericin B refers to using the deoxycholate form. We only found one case of intravesical administration of the liposomal form. This was a 65-year-old female patient who developed septic shock of abdominal origin, with isolation of *C. parapsilosis* in blood culture, urine culture and surgical wound. As part of the treatment of candiduria, bladder irrigation with liposomal amphotericin B was used continuously for three days at a concentration of 50 mg/l with a good outcome.[9] Our case is the first to be described in a paediatric patient.

Intravesical administration of amphotericin B deoxycholate is more effective using continuous rather than intermittent irrigation. The most commonly used concentration is 50 mg/l, although a higher concentration is used in intermittent administrations.[10] In our case, intermittent instillations of liposomal amphotericin B were administered as the urinary catheter had only one lumen, and catheter replacement was difficult. The concentration we used was effective in intermittent irrigation with amphotericin B deoxycholate[10] and was used for five consecutive days with good results.

We would conclude that intravesical instillation of liposomal amphotericin B may be a safe alternative in treating azole-resistant *Candida* species cystitis. However, more extensive studies are needed to support this assertion.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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