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Brief Report

Community Surveillance of MRSA and *Staphylococcus aureus* in Rural Portugal: The BI-STAPH Project—Phase 1: Sertã

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Abstract

Introduction: Colonization by *Staphylococcus aureus*—including methicillin-resistant strains (MRSA)—represents a growing public health concern, particularly in community and rural settings. In Portugal, limited data are available regarding its prevalence in populations with agricultural or animal-related exposures. Objectives: To determine the prevalence of *S. aureus* and MRSA nasal colonization among adults residing in the municipality of Sertã, Portugal, and to explore potential sociodemographic and behavioral factors associated with colonization. Methods: A cross-sectional study was conducted with 292 adult participants from multiple parishes of Sertã. Nasal swabs were collected for microbiological identification of *S. aureus* and MRSA. Data on sociodemographic characteristics, occupational exposure, animal contact, and recent antibiotic use were collected via structured questionnaires. Descriptive statistics and inferential analyses (chi-square and Fisher's exact tests) were performed, and odds ratios were estimated. Results: The overall prevalence of *S. aureus* colonization was 19.9% (58/292), with MRSA detected in 4.8% (14/292) of participants, representing 24.1% of all *S. aureus* carriers. Colonization by *S. aureus* was slightly more frequent among females (51.7%) and predominantly observed in individuals aged 35–59 years. MRSA was more frequent in participants aged $\geq$ 60 years and was equally distributed between sexes. 57% of MRSA cases reported recent antibiotic use and all MRSA cases reported daily contact with animals—primarily domestic species. No statistically significant associations were identified between colonization and the analyzed variables, although trends suggested increased risk among individuals with animal contact and moderate to high-risk occupations. Conclusions: This study revealed a notable prevalence of *S. aureus* and MRSA colonization in a rural Portuguese population. Although no statistically significant associations were found, with animal contact, occupational exposure, and recent antibiotic use emerged as relevant epidemiological factors. These findings highlight the need for strengthened surveillance and further investigation into zoonotic transmission and occupational risk in rural environments.

Keywords: *Staphylococcus aureus*; MRSA; nasal colonization; rural population; occupational exposure; antimicrobial resistance

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1. Introduction

Staphylococcus aureus is recognized as one of the major pathogenic agents in humans, responsible for a wide spectrum of clinical infections. This microorganism can cause superficial skin infections, such as furuncles, abscesses, and impetigo, as well as severe invasive infections, including bacteremia, endocarditis, pneumonia, and urinary tract infections. Its ability to colonize the skin and mucous membranes of healthy individuals, combined with the production of various toxins and virulence factors, contributes to its success as an opportunistic pathogen. The emergence and dissemination of antibiotic-resistant strains, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), have exacerbated the clinical impact of this pathogen, complicating effective treatment and increasing the associated morbidity and mortality. This clinical versatility, coupled with its antimicrobial resistance potential, positions *S. aureus* as a growing public health concern in both hospital and community settings [1–7].

Over recent decades, the emergence and global spread of MRSA have become a major public health issue, hindering infection management and contributing to higher morbidity, mortality, and economic costs [8,9]. While initially restricted to hospital environments (HA-MRSA), community-acquired MRSA (CA-MRSA) has increasingly affected non-hospitalized populations [10,11]. The emergence of resistance in *S. aureus* has occurred in successive waves: penicillin-resistant *S. aureus* (PRSA) appeared shortly after penicillin's introduction, followed by methicillin-resistant *S. aureus* (MRSA) in the early 1960s, and more recently, vancomycin-resistant *S. aureus* (VRSA) has been sporadically reported [3,8]. These patterns illustrate the remarkable adaptive capacity of *S. aureus* and the continuous challenges posed to clinical management. Beyond its clinical impact, awareness and knowledge of MRSA among healthcare workers and the general public play a pivotal role in prevention and control strategies. Previous surveys have demonstrated gaps in knowledge and practices, both in the community and among food handlers, highlighting the need for educational interventions [12].

The prevalence of *S. aureus* and MRSA colonization varies considerably across geographic regions and population groups, influenced by sociodemographic factors, underlying health conditions, environmental exposures, healthcare contacts, and personal hygiene practices. Recent studies suggest that age, sex, and certain socioeconomic conditions may influence colonization risk, although the underlying mechanisms remain incompletely understood [13–15].

In the Portuguese context, most studies have focused on large urban centers or hospital settings, with limited data available concerning inland or semi-rural populations [16–18].

Thus, the present study aims to determine the prevalence of *S. aureus* and MRSA colonization among individuals residing in the Sertã region, while simultaneously assessing potential associations with sociodemographic variables such as age and sex. Monitoring the prevalence of these strains in a non-hospitalized population may contribute to a better understanding of the local epidemiology of *S. aureus* and support the development of more effective prevention and control strategies.

The specific objectives are to:

- Analyze the geographic distribution of *S. aureus* within the Sertã municipality;
- Identify sociodemographic and environmental factors associated with colonization and potential dissemination of the bacterium;
- Characterize the main sources and contexts of MRSA dissemination in the community.

Given the global and national impact of MRSA, its potential links to community, occupational, and zoonotic exposures, and the scarcity of rural Portuguese data, this study was specifically designed to investigate MRSA prevalence and associated risk factors in the Sertã region.

2. Materials and Methods

This study was conducted in the municipality of Sertã, located in the Beira Interior region of Portugal. The target population included community-dwelling adults residing across all local parishes. The sample represented approximately 2% of the total estimated population of the municipality, based on official data from the 2021 *Census* [19].

2.1. Inclusion Criteria

- Age $\geq$ 18 years;
- Permanent residence in one of the selected parishes.

2.2. Exclusion Criteria

- Individuals younger than 18 years;
- temporary residents;
- refusal to provide informed consent;
- acute upper respiratory infection at the time of sampling

2.3. Recruitment and Informed Consent Procedures

Participation was voluntary, following the signing of a written informed consent form, which provided a detailed description of the study objectives and procedures. After consenting, each participant completed a sociodemographic questionnaire addressing variables such as age, sex, occupation, smoking habits, and contact with animals.

2.4. Variable Classification

- **Age groups**: 18–34 years, 35–59 years, $\geq$60 years (based on prior studies and the local population distribution).
- **Occupational risk exposure**: grouped into three categories according to potential occupational risk for *S. aureus* transmission, as described in the literature [20–22]. For example, healthcare workers and abattoir staff were considered high risk, teachers and farmers moderate risk, and office-based professions low risk.
- **Antibiotic use:** recent antibiotic use was defined as use within the past 12 months, consistent with previous surveillance studies. We recognize that shorter intervals (e.g., 3–6 months) may better capture selective pressure.
- **Animal contact**: classified as contact with domestic animals, non-domestic animals, or both.

2.5. Sample Collection Procedures

Bilateral nasopharyngeal swabs were collected using COPAN® Amies transport medium swabs (Murritea, CA, USA), ensuring sample viability for up to 48 h at temperatures between 2 °C and 8 °C. Sampling was performed in public locations, adhering strictly to biosafety standards and good laboratory practices.

2.6. Laboratory Processing and Identification of S. aureus and MRSA

Samples were processed following standardized laboratory protocols. Identification of *Staphylococcus aureus* was performed via selective culture on CHROMagar™ *Staph aureus*, followed by confirmation using standard biochemical tests (catalase and coagulase). Detection of MRSA was achieved through incorporation of oxacillin or cefoxitin into the culture media and/or rapid agglutination tests.

The reference strain *S. aureus* ATCC 29,213 was used as a quality control.

2.7. Statistical Analysis

Statistical analysis was conducted using SPSS® software version 30.0 (IBM Corp., Armonk, NY, USA). Chi-square tests were applied to evaluate associations between *S. aureus*/MRSA colonization and sociodemographic variables. Significance level (for type I error) of 5%. A post hoc power calculation indicated that the study was powered to detect prevalence estimates but had limited ability to identify statistically significant associations within small subgroups, such as MRSA carriers

2.8. Ethical Considerations

The study was approved by the Ethics Committee of the University of Beira Interior (approval code: CE-UBI-Pj-2023-054) on 16 January 2024. All procedures complied with the Declaration of Helsinki and adhered to recognized standards of good scientific practice.

3. Results

3.1. Sample Characterization

A total of 292 adult individuals residing in various parishes within the municipality of Sertã participated in this study. Most participants were from the parish of Sertã and the Union of Parishes of Cernache do Bonjardim, Nesperal, and Palhais.

The sample exhibited a slight predominance of females (58.9%; n = 172), while males accounted for 41.1% (n = 120). The age distribution was concentrated primarily in the 35–59 years group, which represented approximately half of the participants.

3.2. Prevalence of Staphylococcus aureus *and MRSA*

The overall prevalence of nasal colonization by *Staphylococcus aureus* was 19.9% (58/292). Among colonized individuals, the distribution by sex was relatively balanced: 30 cases (51.7%) in females and 28 cases (48.3%) in males.

As for methicillin-resistant *Staphylococcus aureus* (MRSA), 14 positive cases were identified, corresponding to a global prevalence of 4.8% (14/292) and representing 24.1% of the *S. aureus* carriers. MRSA cases were equally distributed between sexes, with 7 cases (50%) in males and 7 cases (50%) in females.

Positive cases were identified across most of the parishes studied, with a higher concentration in more densely populated areas. No MRSA cases were detected in the parish of Carvalhal (Table 1).

Table 1. Participant characteristics.

Variable	*n* (%)
Sex (female)	172 (58.9%)
Sex (male)	120 (41.1%)
Age 18–34	70 (23.9%)
Age 35–59	146 (50%)
Age ≥60	76 (26.1%)
Occupational risk: low	n = 80 (27.4%)
Occupational risk: moderate	n = 140 (47.9%)
Occupational risk: high	n = 72 (24.7%)
Animal contact: domestic	n = 110 (38%)
Animal contact: livestock	n = 40 (14%)
Animal contact: both	n = 70 (24%)

3.3. Sociodemographic Analysis

- Despite a predominance of females in the overall sample, *S. aureus* colonization showed a similar distribution between sexes (51.7% in females vs. 48.3% in males).
- MRSA colonization was also evenly distributed between sexes (50% in each).
- The majority of *S. aureus* carriers (51.7%) belonged to the 35–59 years age group.
- MRSA cases were more frequently observed among individuals aged $\geq$ 60 years (64.3%).

3.4. Associated Factors

Smoking habits:

- Reported by 30.8% of the total sample.
- A slightly higher proportion of carriers was observed among smokers (40.3%), though this did not reach statistical significance ($p > 0.05$).

Occupational risk:

- Most *S. aureus* cases (72.4%) occurred in individuals employed in occupations classified as moderate occupational risk.
- MRSA cases were distributed as follows: 14.2% in low-risk professions, 28.6% in moderate-risk, and 57.2% in high-risk occupations.

Contact with animals:

- Daily contact with animals was reported by 75.8% of *S. aureus* carriers.
- All MRSA cases (100%) were found among individuals with daily contact with animals, predominantly domestic animals (89.3%).

Antibiotic use:

- 57% of individuals colonized with MRSA reported recent antibiotic use (within the previous 12 months).

3.5. Statistical Analysis

Chi-square and Fisher's exact tests did not reveal statistically significant associations between sociodemographic or behavioral variables and colonization by *S. aureus* or MRSA ($p > 0.05$). Odds ratio (OR) analysis indicated a non-significant trend toward increased colonization risk associated with daily contact with animals (OR > 2), although confidence intervals included the null value.

3.6. Summary of Main Findings

- Overall prevalence of *S. aureus*: 19.9%
- Overall prevalence of MRSA: 4.8%
- Factors frequently associated with colonization:
    - Daily contact with animals
    - Moderate occupational risk
    - Recent antibiotic use (for MRSA cases)
    - Age $\geq$ 60 years (for MRSA cases)
- No statistically significant associations were identified.

In Tables 1 and 2 we can observe, in a more schematic way, the general characteristics of the population and the distribution by colonization by *S. aureus* or MRSA, respectively.

Table 2. Colonization rates.

Variable	*S. aureus* (%)	MRSA (%)	
Overall prevalence	19.9%	4.8%	Total sample
Female (n = 172)	17.4%	4.1%	Prevalence in subgroup
Male (n = 120)	23.3%	5.8%	Prevalence in subgroup
Age 18–34	15.7%	1.4%	Prevalence in subgroup
Age 35–59	21.9%	4.1%	Prevalence in subgroup
Age $\geq$ 60	19.7%	9.2%	Prevalence in subgroup

4. Discussion

This study assessed the prevalence of nasal colonization by *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) in an adult population residing in multiple parishes of the Sertã municipality, a rural region in inland Portugal. The sample represented approximately 2% of the total population of Sertã [19], ensuring adequate and representative population coverage.

The overall prevalence of *S. aureus* colonization was 19.9%, and MRSA was detected in 4.8% of participants. These values are consistent with those reported in community-based studies across Europe, where MRSA prevalence generally remains below 5%. However, prevalence rates can vary by population characteristics. For instance, studies in older adults have reported MRSA rates above 14% [3,23–25]. The detection of MRSA in this rural population, particularly in association with daily animal contact, suggests environmental and occupational exposure as contributing factors—in line with literature on zoonotic MRSA in agricultural settings [6]. The higher prevalence of MRSA observed in more densely populated areas could reflect differences in healthcare access, antibiotic consumption patterns, or transmission dynamics linked to urbanization. Although exploratory in nature, these finding merits further investigation.

Demographically, most *S. aureus* carriers were within the 35–59-year age group, aligning with previous findings that working-age adults may be more exposed to colonization due to occupational and behavioral factors. In contrast, MRSA was more frequently observed in individuals aged $\geq$ 60 years, potentially reflecting immunosenescence, cumulative healthcare exposure, or prior antibiotic use [2,5].

Despite the female predominance in the sample, colonization by *S. aureus* and MRSA occurred in equal proportions among sexes—50% male and 50% female among MRSA cases. This balanced distribution suggests that, in this population, sex alone may not be a major determinant of colonization, highlighting the importance of contextual and behavioral factors over biological sex.

Daily contact with animals was strongly associated with colonization: 75.8% of *S. aureus* carriers and 100% of MRSA cases reported regular animal exposure, mostly involving domestic animals. These findings underscore the role of animal reservoirs in the persistence and transmission of MRSA in rural communities, consistent with previous reports [18,26,27]. In this study, "daily contact with animals" was broadly defined, primarily reflecting contact with domestic species. We recognize that differentiating livestock from pets, as well as accounting for frequency and occupational vs. domestic exposures, would provide a more detailed understanding of zoonotic transmission risks.

In terms of occupational risk, most *S. aureus* carriers were engaged in jobs classified as moderate risk, and MRSA cases were distributed across all occupational risk levels—with the highest proportion (57.2%) in individuals with high-risk occupations. This suggests that exposure linked to professional activities, especially those involving animals, may

play a significant role in colonization, even outside the classic high-risk settings such as hospitals or intensive animal farming [28–30].

Although statistical tests, including chi-square and Fisher's exact test, were applied, no statistically significant associations were observed between colonization and the sociodemographic or behavioral variables assessed ($p > 0.05$). This may be attributed to the relatively small number of MRSA cases, which limits statistical power, and to the diversity of exposures within the population.

Notably, recent antibiotic use was reported by 57% of MRSA carriers, reinforcing the role of antimicrobial pressure in selecting resistant strains, a mechanism widely described in the literature [31,32]. While not statistically significant in this analysis, the consistent presence of this factor among MRSA cases warrants further attention. The definition of 'recent antibiotic use' as within the past 12 months may overestimate its influence on MRSA selection. Future studies should adopt shorter cutoffs (3–6 months). Finally, combating antimicrobial resistance requires not only epidemiological surveillance but also the exploration of novel therapeutic strategies. Innovative approaches, such as targeting bacterial metallophores as virulence factors and the use of 'Trojan horse' antibiotics to circumvent resistance, represent promising avenues for future research and drug development [33].

This study has several limitations:

- The overall sample size, particularly the small number of MRSA carriers (n = 14), limited the statistical power to detect associations. This limitation must be considered when interpreting the findings. Future research should prioritize larger cohorts and multi-center collaborations to achieve greater statistical robustness.
- Another important limitation was the absence of molecular characterization of isolates (e.g., spa typing, SCCmec analysis). This precluded discrimination between healthcare-associated, community-associated, and livestock-associated MRSA. Future studies should prioritize genetic characterization to clarify strain origin and transmission dynamics.

Future research should prioritize larger and more diverse samples, combined with genetic characterization of isolates (e.g., *spa* typing, *SCCmec* analysis), to better understand strain origin and transmission dynamics, and to inform targeted prevention strategies.

- We did not collect information on participants' comorbidities (e.g., diabetes, immunosuppression) or household size, both of which may influence colonization risk. These variables should be integrated into future community-based studies.

5. Conclusions

This study investigated nasal colonization by *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) among adults residing in a rural community in the municipality of Sertã. The overall *S. aureus* colonization rate was noteworthy, with a particularly significant detection of MRSA carriers within the community.

Although no statistically significant associations were identified, the findings suggest potential links between MRSA colonization and factors such as daily contact with animals, occupations classified as moderate risk, and recent antibiotic use. The predominance of MRSA cases among older adults and the equal distribution across sexes highlight epidemiological patterns that merit further investigation.

Despite the limitation of not having performed molecular typing of isolates, the results underscore the relevance of enhancing MRSA surveillance in rural contexts, where occupational and environmental exposures may facilitate the dissemination of resistant strains. Future studies incorporating genotypic characterization and larger, more representative samples will be essential to clarify transmission pathways, evaluate the role of animals as reservoirs, and guide the design of effective prevention strategies.

Overall, these findings emphasize the urgent need for targeted awareness and preventive interventions—particularly among occupationally exposed populations—as a critical step toward mitigating the risk of MRSA emergence and transmission in community settings.

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Informed Consent Statement: Patient consent was waived as this was a retrospective study, in which there was only one consultation of anonymized data.

Data Availability Statement: Data may be shared, in case of a duly justified request, considering the necessary protection.

Conflicts of Interest: The authors declare no conflicts of interest.

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