

Article

MRSA Colonization in Workers from Different Occupational Environments—A One Health Approach Perspective

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Abstract: *Staphylococcus aureus* and particularly methicillin-resistant *S. aureus* (MRSA) infections are currently associated with extremely high morbidity and mortality rates worldwide. The global escalation in the development of antibiotic-resistant human pathogens and *S. aureus* ability in developing new clones with the capacity to invade community settings, leads to an urgent need to develop accurate and efficient assessments of *S. aureus* colonization in occupational settings, particularly those with increased risk of human and animal colonization and food contamination. Here we present cross-sectional studies with the aim to assemble crucial information regarding MRSA prevalence in workers from five different Portuguese occupational environments (bakeries, swineries (humans and animals), ambulance crews, veterinary clinics and healthcare facilities). Our data demonstrated high prevalence of *S. aureus* asymptomatic carriers among bakery workers (40%; 75% MSSA and 25% MRSA), swinery workers (54%; 8% MSSA and 46% MRSA), firefighters (48.5%; 24% MSSA and 21% MRSA) and healthcare workers (Study 1: 42.2%; 18.4% MSSA and 23.7% MRSA, Study 2: 43.3% MRSA). *S. aureus* prevalence in veterinary staff was 7.1% (MSSA), lower than the results obtained in control groups (33.3% *S. aureus*; MRSA 4% to 10%). The present study sustains the urge to develop accurate and efficient assessment of *S. aureus* human and animal colonization, particularly in high risk occupational settings, with proper guidelines and validated procedures in order to avoid potential hazardous health outcomes associated with bioaerosol exposure and associated infectious diseases.



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Keywords: MRSA; occupational exposure; one health approach

1. Introduction

It is estimated that 20% of the human population are persistent carriers of *Staphylococcus aureus*, and nearly 30% are intermittent carriers [1]. Because it is both a commensal bacterium and a pathogen, colonization can be dangerous, working as a reservoir and leading to the spread of the bacteria or future infections in the colonized individual [2]. Additionally, *S. aureus* is the main agent of nosocomial and community-acquired infections, with high percentages of strains resistant to various antibiotics, antiseptics and disinfectants. It is a common cause of bacteremia and endocarditis, as well as osteoarticular, skin and soft tissue, pulmonary and device related infections [1–3].

Methicillin resistance is a prevalent antibiotic resistance associated with *S. aureus* and healthcare worldwide. MRSA strains produce an altered penicillin-binding protein (PBP2a) that is encoded by an acquired gene (*mecA*) associated with decreased affinity for most semisynthetic penicillins [1]. The emergence of MRSA is due to the acquisition and

insertion of a mobile genetic element, designated staphylococcal cassette chromosome *mec* (SCC*mec*), that carry the *mecA* gene into the chromosomes of susceptible strains [1].

MRSA strains were previously more limited to the hospital environment, but are now showing rising degrees of linkage with community-acquired infections, as well as the emergence of multidrug-resistance [1,3,4]. MRSA has a persistently high mortality rate and may infect nearly any anatomical regions -. The infecting strains match the colonizing strains in 50–80% of MRSA infections, and it is estimated that colonization may increase infection risk by up to 25% [1]. Any item in contact with the skin can serve as a fomite in MRSA transmission, and the bacteria can remain for long periods of time in hosts or the environment, complicating attempts at eradication [1,3]. The spread of resistant strains can make common infectious diseases difficult, sometimes impossible, to treat, and leads to increased medical costs, prolonged hospitalizations and increased mortality [1,5].

Portugal is one of the countries with the highest prevalence of MRSA. Despite a decrease of 8.2 percentage points from 2014 to 2017, the percentage in 2017 was still at 39.2%, and MRSA continues to be defined as a public health priority [6]. The decrease in the prevalence of MRSA is due to the implementation of national recommendations and guidelines, prudent use of antibiotics, and prevention and control of infections [7].

Apart from humans, MRSA colonization and infection has also been reported in companion, livestock and wild animals [1]. In fact, dust is suspected to have an important role in transmission of livestock-associated MRSA between pigs, farmers and farmers' families (Feld et al., 2018). Additionally, the indiscriminate use of antibiotics in animal husbandry and other agricultural activities, along with poor infection control measures, has largely contributed to an increase in the emergence of resistant strains and dissemination among livestock [1]. LA-MRSA has recently attracted considerable attention as a zoonotic risk, especially for people who interact closely with farm animals. The detection and geographic spread of LA-MRSA in the EU/EEA population increased between 2007 and 2013, according to an ECDC survey, highlighting the veterinary and public health relevance of LA-MRSA as a "One Health" problem [8].

Comprehensive MRSA strategies targeting all healthcare settings remain essential to slow the spread of MRSA in Europe. Surveillance for MRSA in animals and food is currently voluntary and only carried out in a limited number of countries [9]. Insufficient infection control and prevention contributes to the rapid progression of antibiotic resistance. As a response, all barriers to the spread of resistance must be determined [10].

To obtain information regarding MRSA prevalence in workers from five different Portuguese occupational environments (bakeries, swineries, ambulance crews, veterinary clinics and healthcare facilities), cross-sectional studies were performed. The studies were integrated into larger studies comprising also the assessment of MRSA contamination in the environment (ambulances) or, in the case of swineries, animals.

2. Materials and Methods

2.1. Workplaces and Workers Assessed

Biological samples were collected from 5 swineries assessing a total of 68 samples; 26 from workers (including veterinarians, engineers and workers) and 42 from animals. Regarding the animals, we selected 42 pigs from the maternities in 3 swineries, and 30 pigs from the stalls (3 weeks old) from 2 swineries, following the procedures published in [11,12]. Moreover, from a veterinary clinic, we collected samples from 14 volunteers (all day shift workers, including veterinarians and auxiliary workers) [13].

Regarding the ambulance crew, we collected 98 environmental samples from the ambulances and 33 from workers (ambulance crew), as previous reported [14].

From healthcare workers (including doctors, nurses, laboratory technicians and auxiliary workers), biological samples were taken in two central hospitals in Lisbon, assessing a total of 68 biological samples; 38 from Hospital 1 and 30 from Hospital 2 [15]. Additionally, 25 biological samples were collected in 10 Primary Health Care Centers (PHCC).

Furthermore, we collected 74 biological samples from workers (including supervisors, bakers and auxiliary workers) in 13 bakeries.

A control group with 55 biological samples was collected from volunteers (mostly from the academic environment, including teachers, students, auxiliary and administrative workers) with no occupational contact with healthcare or animal facilities.

All studies mentioned above were carried out in Portugal. Additional information about the sample collection procedure can be found in Table 1. All volunteers enrolled in the studies were healthy individuals (with no previously diagnosed pathologies). Inclusion criteria considered were adult voluntaries (older than 18 years old and younger than 65 years old) with no acknowledged previously diagnosed pathology of any type, no gender criteria were utilized. Exclusion criteria applied included viral and bacterial infections.

Table 1. Environmental and biological samples collected in each workplace environment.

Occupational Environment/Control Group	Biological Samples	Environmental Samples	References
Swineries (N = 5)	Nasopharyngeal swabs (N = 68; 26 humans and 42 animals)	Not performed	[11,12]
Veterinary clinic (N = 1)	Nasopharyngeal swabs (N = 14)	Not performed	[13]
Ambulance crew (N = 12)	Nasopharyngeal swabs (N = 33)	Surface swabs (N = 98) performed on floor, gurney handle, chairs, entrance and ceiling handle, washstand, shelves, driver's cabin (wheel) and air exit of the medical cabin	[14]
Healthcare Environment (N = 3)	Nasopharyngeal swabs (N = 93; 38 from Hospital 1, 30 from Hospital 2, 25 from PHCC)	Not performed	[15]
Bakeries (N = 13)	Nasopharyngeal swabs (N = 74)	Not performed	Data not published
Control group (N = 2)	Nasopharyngeal swabs (N = 55; 25 from Study 1, 30 from Study 2)	Not performed	Data not published

2.2. Samples Collection

All the biological samples were obtained through nasopharyngeal swab procedure for *S. aureus* identification, using transport swabs with Stuart media, and immediately transported to the laboratory. The swab was inserted into the nostrils (one at a time), and moved straight back along the floor of the nasal passage until it reached the posterior wall of the nasopharynx (about 4 to 6 cm or 1.6–2.5 inches), was gently rotated for a few seconds and carefully removed without touching the sides of the nostrils. All workers provided a signed written informed consent before enrolment in the study, making sure all the inherent ethical principles were properly safeguarded.

The projects were submitted and approved by Escola Superior de Tecnologia da Saúde de Lisboa Ethical Council (Lisboa, Portugal) (Re: CE-ESTeSL-N° 63-2019; CE-ESTeSL-N° 18-2019). The studies are in accordance with the Helsinki Declaration and Oviedo Convention and in Agreement with the Portuguese law n° 58/2019 of 8 of August regarding data protection.

Surface samples were collected by swabbing the surfaces using a 10 × 10 cm square stencil, which was disinfected with a 70% alcohol solution between each sampling (ISO 18593, 2004). Following inoculation, each swab was later extracted with 1 mL of 0.1% Tween™

80 saline solution (NaCl 0.9%) for 30 min at 250 rpm in an orbital laboratory shaker (Edmund Bühler SM-30, Hechingen, Germany) [14].

2.3. Staphylococcus Aureus Identification

For *S. aureus* identification, the biological (N = 337) and environmental swab samples (N = 98) were inoculated in Columbia agar, with 5% sheep blood and CHROMID® MRSA, then incubated for 24 and 48 h at 37 °C. Suspicious colonies were isolated and identification performed through a catalase test, using a Slidex Staph Kit (Biomerieux ref #73115) and Slidex MRSA detection Test Kit (Biomerieux ref #73117). In this work, positive (MRSA laboratory collection) and negative (*S. aureus* ATCC 25923) control strains were included as positive and negative controls.

3. Results

3.1. Swineries

S. aureus was detected in 54% of the 26 workers selected from 5 swineries, 8% of the workers were colonized with MSSA and 46% with MRSA. The prevalence of MRSA was 34% in the 42 pigs selected from maternities in 3 swineries and 66% in 30 pigs selected from stalls in 2 swineries. Swineries where the colonization by MRSA in animals was higher also demonstrated higher colonization in workers (Figure 1).

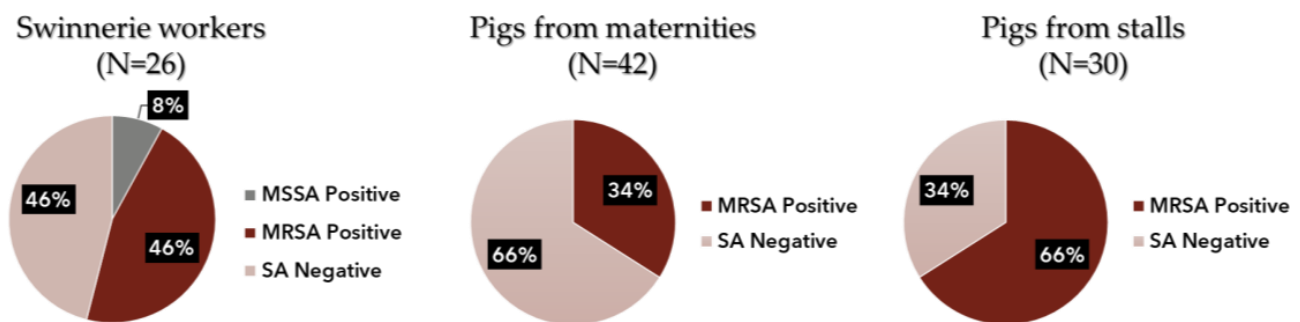


Figure 1. MRSA prevalence in the swineries.

3.2. Veterinary Clinic

The prevalence of *S. aureus* in the 14 workers from the veterinary staff was 7.1% (1). The identified *S. aureus* strain was susceptible to methicillin (MSSA) (Figure 2).

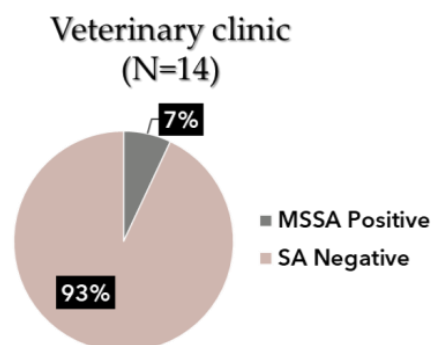


Figure 2. MRSA prevalence in the veterinary clinic.

3.3. Ambulances

The prevalence of *S. aureus* detected in the 98 environmental samples swabs collected in 12 ambulances from two fire stations was 3%, with only one colonized with MRSA. Of the 33 firefighters who participated in the study, 48.5% were colonized with *S. aureus*, 24% MSSA and 21% MRSA (Figure 3).

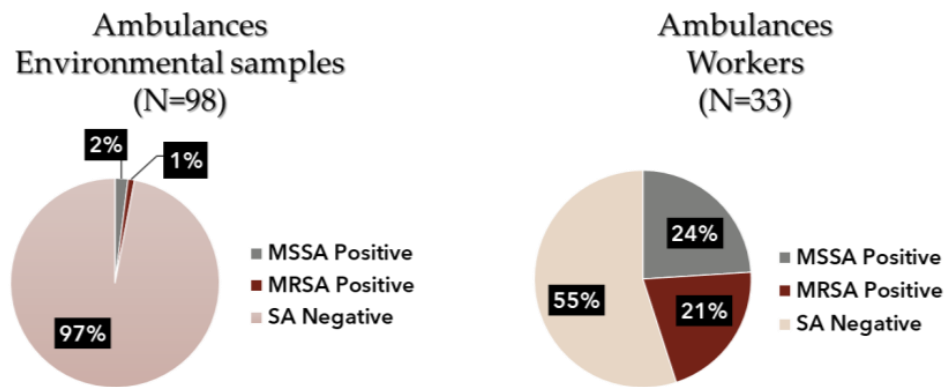


Figure 3. MRSA prevalence in the ambulances and crew.

3.4. Healthcare Environment

In the study carried out at Hospital 1, 42.2% of the 38 healthcare workers were colonized with *S. aureus*, of which 18.4% were MSSA and 23.7% MRSA. The prevalence of MRSA at Hospital 2 was 43.3% in a population of 30 healthcare workers. In the study carried out at Primary Health Care Centers, no MRSA was detected from the nasal swabs of the 25 workers that participated (Figure 4).

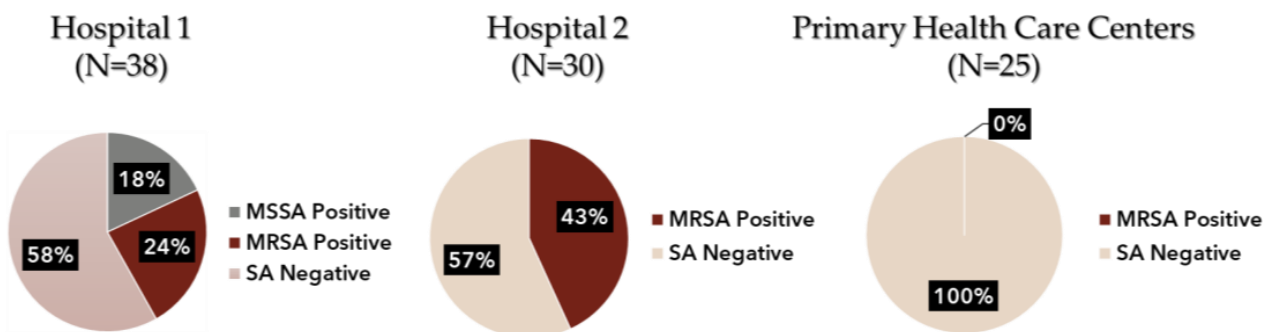


Figure 4. MRSA prevalence in the healthcare workers.

3.5. Bakeries

In the assessed bakeries, we identified a 40% prevalence of asymptomatic *S. aureus* carriers among the workers, of which 75% were sensible to methicillin (MSSA) and 25% presented a resistance phenotype (MRSA). Overall MRSA was found in 10% of the analyzed samples (Figure 5).

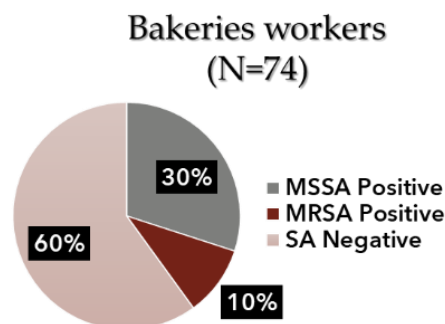


Figure 5. MRSA prevalence in the bakery workers.

3.6. Control Group

From 55 healthy volunteers without regular contact with the healthcare setting analyzed in the two studies, the prevalence of MRSA was 7%. Study 1, with 25 samples

collected, had one MRSA strain (4%). Study 2, with 30 samples collected, identified 33.3% volunteers colonized with *S. aureus*, 23.3% were MSSA and 10% MRSA (Figure 6).

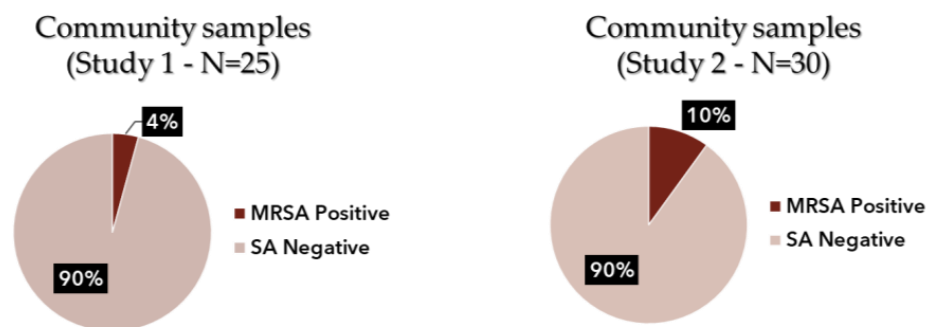


Figure 6. MRSA distribution in the control groups.

4. Discussion

Direct contact with pigs in swine occupational environment is a recognized risk factor for LA-MRSA colonization and swine workers, due to their daily work activities, are expected to be particularly highly exposed. In addition, veterinarians also have a significantly elevated risk of becoming LA-MRSA carriers [13,16,17]. In our study a higher prevalence was found in swine workers, but not in the veterinarians studied. This could be due to the fact that besides direct contact with infected animals occurring in both occupational environments, exposure to bioaerosols has also been suggested as a determinant for nasal carriage of LA-MRSA in swine workers [18], due to the dust present in swine [13,19]. In fact, during pig's activity, emission of LA-MRSA may occur from mucus or by abscess of skin particles, and therefore bioaerosols can be released and disseminated in the stable air [20]. In addition, bioaerosols and dust may also constitute a source of transmission to humans outside the swine, due to emissions outside the swine or indirectly by contamination of workers cloths, tools, etc., which are brought out from swine facilities [21]. This could justify the increasing number of people without direct contact to livestock being registered as LA-MRSA positive [22–25], and also the results of our control groups.

To the best of our knowledge, this study is the first one reporting data concerning MRSA nasal carriage in bakery workers. As in the animal production setting, this occupational environment is prone increased exposure to flour dust and bioaerosols [26,27] and, consequently, the carriage of *S. aureus* and MRSA. Indeed, the dust can serve as a vehicle for microorganisms to workers respiratory airways, boosting workers exposure [13].

Data analysis revealed that healthcare occupational exposure, including ambulance crews, is concerning high, following the trend already reported [6]. Previous studies carried out in Portugal emphasize that the main mode of transmission of MRSA is through the hands, with the absence of proper hand hygiene being the most common mode of transmission [28]. Thus, since health professionals are in direct contact with patients who may be contaminated, non-compliance with hygiene rules can be a way of spreading MRSA inside and outside the hospital environment, and in the community [29]. As previously mentioned, *S. aureus* has the ability to colonize different areas of the human body, with a preference for the nasopharynx [30] and the ability to spread as well as being transmitted by direct contact (mainly by hands) or indirect (contaminated surfaces) [31–33]. Indeed, one of its fundamental biological characteristics is the ability to colonize the healthy population asymptotically (asymptomatic carrier), thus assuming an important role in spreading to other areas of the body, to other people and even contaminating food and surfaces during handling [31–33]. This colonization is considered a risk factor for the onset of infections by *S. aureus*, often combined with methicillin resistance-MRSA, increasing the risk of clinical disease [33,34].

The contrasting prevalence of MRSA colonization reported in each of the occupational environments assessed might be attributed to differences in hygiene practices and workers health education, both of which have a role in reducing MRSA contamination [35–37].

Another aspect to consider is *S. aureus* host-specificity, as companion animals treated at veterinary clinics are not frequently colonized by *S. aureus*, in contrast to meat-producing animals [38]. Thus, our results sustain the prerogative that exposure to bioaerosols at workplaces can represent a health hazard and potentially result in infectious disease [39], which is concerning both for workers and for the spread of these microorganisms in the community.

Staphylococcus aureus is reported as being a very robust species, which is highly resistant to environmental stress (e.g., desiccation) [40]. On surfaces, a persistence of 7 days to 7 months was reported [41] justifying the surface swabs as a sampling method to assess this species distribution, as was done for the ambulances' surfaces. In the performed studies, environmental sampling was not in the aim and objectives of bakeries, swineries or healthcare facilities studied; however, the prevalence of *S. aureus*, including in the isolation of MRSA, reveals that dust is, in fact, a source of contamination and therefore, environmental sampling must be further included in colonization assessments. Furthermore, other sampling methods such as air and settled dust were already employed in different studies [16,18,21,42,43] corroborating the need to increase the protocol concerning the sampling approach to assess MRSA contamination in workplaces.

In order to provide more information regarding *S. aureus* colonization levels among workers from high risk occupational settings (of both human and animal colonization as well as food contamination), further studies should increase the sample number of assessed locations should be increased in future studies as well as different locations regarding the same occupational setting, and carry out continuous assessment over time. Furthermore, molecular biology methods could also be performed to assess clone origin (HA-MRSA; CA-MRSA; LA-MRSA) and enhance information regarding colonization origin.

5. Conclusions

Our results clearly sustain the need to develop accurate and efficient assessments of *S. aureus* for both human and animal colonization, particularly regarding MRSA, with proper guidelines and validated procedures. Considering *S. aureus* dissemination in the community, and the fact that it has the capacity to colonize asymptotically (asymptomatic carrier) and spread to different areas of the body, other individuals and even food and surfaces during handling, the assessment of *S. aureus* in high risk occupational settings (including healthcare settings, animal productions and food handling) is crucial to avoid potential hazardous health outcomes associated with bioaerosols exposure including associated infectious diseases.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Escola Superior de Tecnologia da Saúde de Lisboa (Re: CE-ESTeSL-N° 63-2019; CE-ESTeSL-N° 18-2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Through the UIDB/05608/2020 and UIDP/05608/2020.

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

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