Nasal carriage of Staphylococcus aureus in livestock in North of Morocco

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Staphylococcus aureus, nasal carriage, animals, breeders, MRSA, Morocco.
Abstract
Background: In Morocco, data of LA-MRSA nasal carriage are still limited. The objectives of this study were to determine for the first time the nasal carriage rate, antimicrobial susceptibility profiles and virulence genes of S. aureus isolated from animals and breeders in close contact. Methods: From 2015 to 2016, 480 nasal swab samples were collected from 27 different Livestock areas in Tangier. The antimicrobial susceptibility phenotypes were determined by disk diffusion according to EUCAST 2016. The presence of nuc, mec A and his homologue mec C, lukS/F-PV, and tst genes were determined by PCR for all isolates. Results: The overall S. aureus nasal carriage rate was low in animals (9.97%) and high in breeders (60%) with a statistically significant difference, (OR = 14.321; 95% CI = 7.484-27.405; p< 0.0001). In general, S. aureus strains were susceptible to the majority of antibiotics and the higher resistance rates were found against tetracycline (16.7% in animals and 10% in breeders). No MRSA was detected in animals and breeders. A high rate of tst and lukS/F-PV genes has been found only in animals (11.9% and 16.7%, respectively). Conclusion: Despite the lower colonization rate of S. aureus and the absence of MRSA strains in our study, S. aureus strains harbored a higher frequency of tst and lukS/F-PV of virulence genes, which is associated to an increased risk of infection dissemination in humans. This highlights the need for implementing adequate approaches for prevention. Further larger and multi-center studies are needed to validate and confirm our findings. Keywords: Staphylococcus aureus, nasal carriage, animals, breeders, MRSA, Morocco

Background
Nasal carriage was been shown to be a predisposing factor for infections followed by inter individual transmission and dissemination in different environments [1]. The emergence of methicillin-resistant S. aureus strains (MRSA) is linked to the acquisition of the mecA gene [2] or its mecC variant encoding a variant of the Penicillin-Binding Protein (PBP) gene [3]. MRSA appeared firstly in a hospital in the early 1960s [4]. Since then, it has been established around the world as an endemic hospital pathogen (Hospital Acquired-MRSA: HA-MRSA). The late 1990s and 2000s saw the emergence and worldwide spread of particular strains of this
microorganism known as Community-Acquired MRSA (CA-MRSA) [5]. CA-MRSA strains are different from typical hospital strains in many ways including, in terms of clinical expression, toxic and genotypic characteristics [5]. Over the past two decades, MRSA has emerged in a variety of animal populations, mainly in pigs and veal calves [6], but also in pets and horses [7]. These strains known as Livestock associated MRSA (LA-MRSA) strains had a genetic origin different to that of human strains previously described with isolates mainly belonging to clonal complex CC398 [8]. In the last decade, MRSA clones with a divergent mecA homolog, named mecC (formerly mecALGA251), have been detected in different animal species and also humans [3].

In African countries, studies about colonization of LA-MRSA seem to be scarce. The first report of ST398 in humans in Africa was described by Elhani et al [9] who isolated one MRSA-ST398-t899 in the nasal sample of a farmer in Tunisia. In Morocco, a study conducted by Mourabit et al [10] revealed that the nasal carriage colonization rate of MRSA was 1.4% in humans. Interestingly, this work identified for the first time one isolate characterized as ST398-MRSA-IV spa t011 that exhibited multidrug resistance and was not Smal typeable.

The objective of this work was to determine the nasal carriage of S. aureus, and the antimicrobial resistance and virulence genes in healthy farm animals and their breeders in Tangier.

**Methods**

**Isolation and identification of nasal S. aureus isolates**

Livestock sampling areas included 16 small farms, one stable (unique public equestrian center of Tangier, which provides trained horses and 10 local sheep and goat farms in different parts of Tangier. In general, the preference for selection of sampling sites was given to small farms or premises near the most populated areas of the city. This study was carried out between 2015 and 2016 and focused on clinically healthy animals and volunteers in close contact with these animals. No antibiotics were used on them during the previous month. Likewise, none of the volunteers had any chronic illness during the sampling period, nor had they used any antibiotics or had been hospitalized in the last three months.

A nasal swab of both nostrils was done simultaneously for each of the animals and volunteer
breeders.

Consent and ethics approval: As the majority of breeders were illiterate, informed oral consent was obtained from all participants following the explanation of the objectives of the study and this was approved by our institution. Confidentiality of the participants was maintained using unique code.

**Phenotypic and molecular identification**

To increase the chances of isolating the strains, the nasal samples were grown on BHI liquid and then incubated at 37°C for 24 hours. The isolation was carried out by successive subcultures on Chapman medium (Biorad) and on chromogenic media (Biorad), then incubated for 24 to 48 hours at 37°C. Suspicious colonies were identified as *S. aureus* by colony morphology, catalase, and Dase tests. Presumptively *S. aureus* were confirmed by PCR for 16S rRNA and *nuc* genes, as previously described [2]. Susceptibility testing was performed with the disk diffusion method according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing [11] for cefoxitin (30 μg), erythromycin (5 μg), lincomycin (15 μg), pristinamycin (15 μg), ciprofloxacin (5 μg), gentamicin (10 μg), tobramycin (10 μg), kanamycin (30 Ui), tetracycline (30 Ui), rifampicin (30 μg), chloramphenicol (10 μg), cotrimoxazole (23.75 ± 1.25 μg) and fusidic acid (10 μg). Inducible or constitutive lincomycin resistance was determined by the double disk diffusion test (D-test). To avoid underestimating the prevalence of methicillin resistance, the presence of the *mecA* and *mecC* gene was determined by PCR in all these isolates, as previously described [2, 3]. All of the *S. aureus* isolates have been tested for the presence of lukS/F-PV and *tst* genes by PCR [12]. *S. aureus* strains ATCC 43300 (*mecA*-positive), ATCC 2011S359 (*mecC*-positive), ATCC 29213 (*nuc*-positive), MW2 (lukS/F-PV -positive) and FRI913 (*tst*-positive) were used as positive controls for PCRs.

All phenotypic and molecular test were carried out in the Biotechnology and Biomolecule Engineering Research Laboratory.

**Statistical analyses**

Comparisons between proportions were drawn with Fisher’s exact test and the Chi-squared test. Odds ratio (OR) and 95% confidence intervals (CI) were also calculated. Differences showing a p value <0.05 were considered significant. Calculations were performed using SPSS version 20.0 software.
Results

**Prevalence of S. aureus in farm animals**

A total of 480 different animals (wild Boar, horses, cattle, sheep and goats) and 50 breeders were nasally screened. Phenotypic and molecular study has permit to isolate 42 (9.6%) and 30 (60%) strains from animals and breeders, respectively. *S. aureus* has only been identified in farm animals (goats, cattle and sheep). None of the 16 horses and the six wild Boar were found to be carriers of *S. aureus*.

**Sensitivity to antibiotics**

Thirty-three *S. aureus* strains in animals and breeders had no resistance (78.5%). All isolated strains were susceptible to cefoxitin, vancomycin, ciprofloxacin, trimethoprim/sulfamethoxazole, tobramycin, gentamycin and chloramphenicol. The higher resistance rates were found for tetracycline (16.7% and 10%) and erythromycin (11.9% and 10%) in animals and breeders, respectively (Table 1). The search for the mecA and the mecC genes did not identify any MRSA strain.

**Exotoxin search**

Molecular tests based on multiplex PCR research of genes coding for Panton Valentin Leucocidin toxin (PVL) and Toxic Shock Syndrome Toxin (TSST-1), has permit to identify in animals seven (16.7 %) strains producing PVL toxin and five (11.9 %) strains producing the toxin TSST-1. However, in breeders no strains carrying the *pvl* gene or *tst* gene have been identified.

**Discussion**

In Morocco, livestock represents a large share of the Gross Domestic Product, which is between 25 and 30% according to the Ministry of Agriculture, Fisheries, Rural Development, Water and Forests [13]. This activity, which still plays an important socio-economic role, involves nearly 70% of the rural population [13]. However, animal farming also develops around urban centers where several farms with less than 20 to 60 heads or more are located in working-class areas or on the outskirts of the city (Figure 1). These animals are housed in premises of different nature and surface. On the social level, livestock in urban areas contributes to income generation and coverage of milk and meat needs of a local layer of the urban population. Livestock feeding consists mainly of market vegetable waste,
pieces of bread left by people or corn. Animal health was not controlled and antibiotics may be overused which increase the risk of human infection, which is even more pronounced if breeding conditions were not suitable (non-vaccination, bad atmosphere conditions in the buildings...).

In Tangier no data of LA-MRSA nasal carriage has been reported yet. Our results revealed statistically lower prevalence of colonization by *S. aureus* in animals compared to breeders (OR = 14.321; 95% CI = 7.484-27.405; p < 0.0001). Furthermore, we showed that all strains were MSSA. Unfortunately, we could not compare our results to Moroccan studies due to the lack of such data at the national scale. In a report from Tunisia including 261 healthy animals, a relatively lower rate of *S. aureus* (6.5%) was reported with different rates depending on animal species. Similar to our findings, this group showed that all *S. aureus* isolates were MSSA [14].

In contrast, some western studies reported various MRSA strain rates [15,16,17]. A Dutch study reported a MRSA colonization rate of 32% in calf-contact persons and 32% in hospitalized patients with contact with pigs and calves [15]. These rates are concerning, given that the prevalence of colonization in the general Dutch population did not exceed 1% [18]. In Switzerland, the pathogen was isolated from only 1% of calves and 0.3% of adult cattle [16], whereas in Denmark, the rate exceeds 64% in goats [17]. Moreover, a recent study conducted by Dweba et al [19], in South African livestock production systems identified MRSA isolates in 27% with a significant relationship (p < 0.001) with the host animal.

In our study, none of the 16 horses were found to be *S. aureus* carrier, whereas only one monitor was colonized by a multi-sensitive *S. aureus*. In fact, this could be due to the low number of volunteer participants who were recruited from one equestrian center in Tangier. Some studies from Europe and Canada identified colonization rates up to 7.9% in horses on farms or in veterinary hospitals [20, 21]. We did not identify any significant differences in antimicrobial resistance between LA-strains and human isolates (p>0.05) (Table 1). The higher resistance rates were found against tetracycline; 16.7% in animals and 10% in breeders. Indeed, resistance to tetracycline and erythromycin is widely reported in the world [22]. These important rates, could be explained by the over-use of these antibiotic in humans as well as in animals [23] which may cause the selection of resistant mutants.
strains and afterwards their dissemination between different species [24]. This use of tetracycline and other antibiotics has become a major global burden for the healthcare system [22]. Substantial efforts were made by World Health Organization and The Food and Drug Administration (FDA) in order to overcome the spread of antibiotics resistance and protect the global health and food security, as well as to optimize the overall use of antibiotics both in humans and animals through the implementation of one-health approaches [22].

In this study, animal strains harbored more virulence genes than human ones. The detection of LA-SA PVL and TSST positive is relevant due to the important role that these toxins seem to play in serious infections [25, 26], and due to the possibility of spread between animal-resistant strains and humans and the environment [25]. Similar strains from Healthy Farm Animals and Pets have been previously reported [14, 27].

To the best of our knowledge, this is the first study carried out in Morocco in order to determine nasal colonization rate of *S. aureus* and MRSA in animals and breeders in Tangier, as well as to study the antibiotic resistance profile and the virulence genes of the isolates. The current study shows several limitations. The nasal carriage of *S. aureus* could be transient and therefore some cases may go unnoticed at the time of our investigation. Furthermore, even though the nasal cavity is the most common site for the carriage of *S. aureus*, other unexplored sites including the extra nasal ones could also host this germ. The limited size of the sample is another limitation of our work, which probably influenced the statistical results.

**Conclusion**

Our results clearly indicate the presence of *S. aureus* colonizing animals, which are used for food production. However, no MRSA has been isolated from these animals, which hinders the ability to obtain an overview of MRSA prevalence in the animal population in Tangier. In addition, we revealed that *S. aureus* strains colonizing animals share some resistances with human strains, enhancing thus the spread and persistence of resistant strains, which represent a potential risk to human and animal health. This risk is further increased by the presence of the two genes *tst* and *pvl*. This highlights the need for raising awareness about strain dissemination across different hosts “One Health concept”.

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Further larger and multi-center studies are needed to validate and confirm our findings. Interestingly, molecular typing of the MSSA strains, *pvl* and *tst* positive strains seems to be of paramount importance.

**Declarations**

**Ethics approval**: Our study was submitted and approved by the internal committee.

**Consent to participate**: As the majority of breeders were illiterate, informed oral consent was obtained from all participants following the explanation of the objectives of the study and this was approved by our institution. Confidentiality of the participants was maintained using unique code.

**Data Availability statements**

All data used to support the findings of this study are available from the corresponding author upon request.

**Conflict of interests:**

No conflict of interests is declared.

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**Author Contributions**

MN designed, organized, collected the nasal samples and wrote the first draft of the paper. MB and AA performed data analysis and interpretation. ZZ and JB drafting the work and revising it critically for important intellectual content. AL designed the study, helped with data analysis and revised the manuscript. All authors read and approved the final manuscript and were responsible for all aspects of the work.

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Table 1
Table 1. Comparison of antimicrobial susceptibility profiles of isolates

|                | Animals n=42 (%) | Breeders n=30 (%) | p-value | OR (95% CI) |
|----------------|------------------|-------------------|---------|-------------|
| kanamycin      | 0 (0)            | 1 (3.3)           | 0.41    | NA          |
| tobramycin     | 0 (0)            | 0 (0)             | NA      | NA          |
| gentamycin     | 0 (0)            | 0 (0)             | NA      | NA          |
| Erythromycin   | 5 (11.9)         | 3 (10)            | 0.55    | 0.822 (0.181-3.740) |
| Ciprofloxacin  | 0 (0)            | 0 (0)             | NA      | NA          |
| Tetracycllin   | 7 (16.7)         | 3 (10)            | 0.32    | 0.556 (0.131-2.351) |
| cotrimoxasol   | 0 (0)            | 0 (0)             | NA      | NA          |
| Chloranphénicol| 0 (0)            | 0 (0)             | NA      | NA          |
| Fusidic-acid   | 1 (2.4)          | 3 (10)            | 0.19    | 0.566 (0.450-46.112) |

OR, odds ratio; CI, confidence interval; NA, not applicable (cannot be calculated due to zero cell)
Figure 1

Distribution of farms residents and breeders