

Interspecific Epidemiology of MRSA in Pig Farming

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Abstract

The populations of MRSA are classified in relation to their origin, distinguishing *S. aureus* Healthcare-Acquired aureus (HA), Community-Acquired (CA) and Livestock-Acquired (LA). For LA-MRSA livestock animals have an important epidemiological role. This has raised the suspicion that the intensive husbandry may be, for the frequency and intensity of use of antibacterial treatments, an elective field of clonal selection of antibiotic resistance. The presence of LA-MRSA in pig production is sure in many countries and the pig is considered a reservoir for transmission to humans and other animals. In fact, farmers and production workers have a higher rate of colonization than the rest of the population. This work reports the presence of MRSA in slaughter pigs (2.3% positivity of the tonsils), sows (10% positivity of nasal swabs) and veterinarians employed in pig production (25% of nasal carriers). Many of the isolates from pigs and man belong to the same genomic patterns.

Keywords: MRSA; Epidemiology; Pig; Veterinarian

Introduction

The first resistant clones of *Staphylococcus aureus* were reported shortly after the introduction of methicillin into clinical practice, but until 1980 their relevance was low [1]. Only later, Methicillin-Resistant *Staphylococcus aureus* (MRSA) was included among the principal agents of nosocomial infections in the world.

Today, in the United States and Europe, up to 30-50% of *Staphylococcus aureus* responsible for hospital infections are methicillin-resistant [2,3] while in the Netherlands and Scandinavia resistance rates are below 3%, due to prevention and properly managed control plans.

The populations of MRSA are now classified on the basis of origin and/or increased circulation, thus distinguishing Healthcare-Acquired (HA-MRSA), Community-Acquired (CA-MRSA) and Livestock-Acquired (LA-MRSA). For the latter, the food-producing animals have an epidemiologically decisive role, and this has led to the hypothesis that intensive animal husbandry may be, because of the frequency of use of antibacterial drugs, an elective field of clonal selection of antibiotic-resistance.

Confirming this indication, a Dutch report showed a much higher rate of colonization in the farmers and the production workers, compared to the rest of the population [4], other subsequent studies have confirmed this indication in the same Holland [5-7] in Belgium [8], Canada [9] in Germany [10] and the United States [11].

The presence of LA-MRSA in pig production is now given in many countries and the pig is now considered a potential reservoir for transmission to humans and other pets. Its most likely epidemiological features would be the selection of LA variants or even the preservation and dissemination of CA variants usually circulating in man [12,13].

The contact with pigs is therefore considered an important risk factor for Livestock Acquired MRSA infections [14,15] and in some countries the problem has even hired social significance: in the Netherlands, the prevalence of ST398 pathotype increased from 0% in 2002 to over 21% in 2006 [7]. The occurrence is associated with the continuous contact with animals, particularly as regards the colonization of veterinarians, farmers and their families.

Risk factors for MRSA infection in pig production have been slightly investigated. The work in continuous contact with pigs is critical, but the quality of health care management can be a factor of amplification or reduction.

The investigations on the localization of MRSA in veterinarians indicated a relatively high prevalence, always upper than the general population. The mapping of bacterial clones confirmed the hypothesis of occupational exposure in horse medicine and food inspection [16,17] while nothing similar has been done in veterinarians working in pig farming.

We therefore decided to investigate the presence of MRSA in pigs (sows in the farm and pigs in the slaughterhouse) and in veterinarians who have a more or less continuous attendance of farms and slaughterhouses.

For a better epidemiological understanding we assessed the genomic similarities between the isolates, separating them into patterns to see if the same genotype can be present in pigs and humans, and if the movement between the two species, favored by the production system is possible.

Materials and Methods

The study was performed on 28 veterinarians, whose professional activity is carried out exclusively in the pig practice. The sample included swine practitioners, veterinarians working on behalf of pig industry Companies and colleagues of the Public Veterinary Service

operating in the slaughterhouses. The sampling took place, after informed consent, by unilateral nasal swab.

Sampling procedures were performed in compliance with Article 13 of Italian Legislative Decree No. 106 of 30 June 2006 (Code relating to the protection of personal data). Everyone has signed two modules, providing informed consent to sampling and to the processing of personal data. The project was approved by the Ethics Committee of the University of Milan.

The sampling of pigs was instead performed in three different slaughterhouses in northern Italy. Ten slaughter batches of heavy pigs from farms in Piedmont and Lombardy were examined. The palatine tonsils (486 pigs) were sampling in the dissection chain and placed in a sterile disposable container. For each slaughter batch, 50% of the pigs was collected and all samples from the same batch of slaughter were kept together.

In two farrow to finish farms, respectively located in the provinces of Cremona and Pordenone, for each of them nasal swabs were performed in 30 sows in the delivery room.

All nasal swabs were performed using a disposable sterile swab with transport medium Ames W/OCH (Oxoid Italy). The sampling was performed in an aseptic and not traumatic manner, avoiding any contaminating contact. Swabs were immediately refrigerated (4-8°C) and under these conditions delivered within 24 hours to the analysis laboratory.

Samples of animal origin have been delivered to the laboratory of Swine Pathology of the Department of Veterinary Sciences and Public Health, University of Milan, while the samples of human origin have been delivered to the Microbiology Virology and Diagnostics Laboratory of University Hospital "Luigi Sacco" of Milan.

The bacteriological protocol was the same, regardless of the origin of the samples and can be outlined as follows.

Nasal swabs

- Direct inoculation on MSA2 Gélose Chapman2 Agar (Biomerieux, France) and on selective Oxacillin-Salt Screen Agar (Biolife, Italy).

- Overnight incubation at 37°C.

- Reading and interpretation. MSA2: the coagulase-positive staphylococci (*S. aureus*) produce colonies with a yellow halo, while coagulase-negative colonies are white and do not involve color change of the medium. Salt Oxacillin Screen Agar: strains of staphylococci that grow on this medium have to be considered oxacillin-resistant (methicillin resistance is extensible to nafcillin). The evaluation of growth on both media allows to detect *S. aureus* (MRSA).

- The isolates were frozen at -20°C in Nutrient Broth with 15% glycerol for a week and then replicated on Tryptic Soy Agar (Biomerieux, France) before the Biomolecular survey.

Tonsils (from slaughtered pigs)

- Homogenization by Stomacher (PBI Italy) after 1:10 preparation in saline (weight/volume).

- Seeding, identification and preparation for biomolecular identification are identical to that applied to nasal swabs.

Molecular Biology

The methicillin-resistant strains of *Staphylococcus aureus* were analyzed by rep-PCR (DiversiLab System - Biomerieux, France). Through this technique similar isolates were classified in different genotypic patterns.

The technique involves three steps: extraction of bacterial DNA (using UltraClean Microbial DNA Isolation kit - Biomerieux, France), rep-PCR amplification and detection of DNA fingerprinting by electropherogram. Through a dedicated software (DiversiLab - Biomerieux, France) and proceed to the comparison of the fingerprints of different samples and their cataloging in the pattern.

The fingerprints of the different samples were then compared using special software (DiversiLab - Biomerieux, France) and finally classified into patterns.

Results

The available results are organized in Table 1. They concern the isolations from nasal swabs of sows and from tonsils of slaughter pigs, in addition to those from nasal swabs performed on veterinarians professionally involved in the pig industry.

| Sampled subject | Sample | N. of samples | Positive | Negative | % Positive |
|-----------------|------------|---------------|----------|----------|------------|
| Veterinarians | Nasal Swab | 28 | 7 | 21 | 25 |
| Sows | Nasal Swab | 60 | 6 | 54 | 10 |
| Slaughter pigs | Tonsils | 486 | 11 | 475 | 2,3 |

Table 1: Categories, diagnostic materials and positive samples

The DiversiLab results are reported in Figure 1 and organized in Table 2. The system allows to check the level of genomic affinity between the isolates, dividing them into "pattern" according to their similarity.

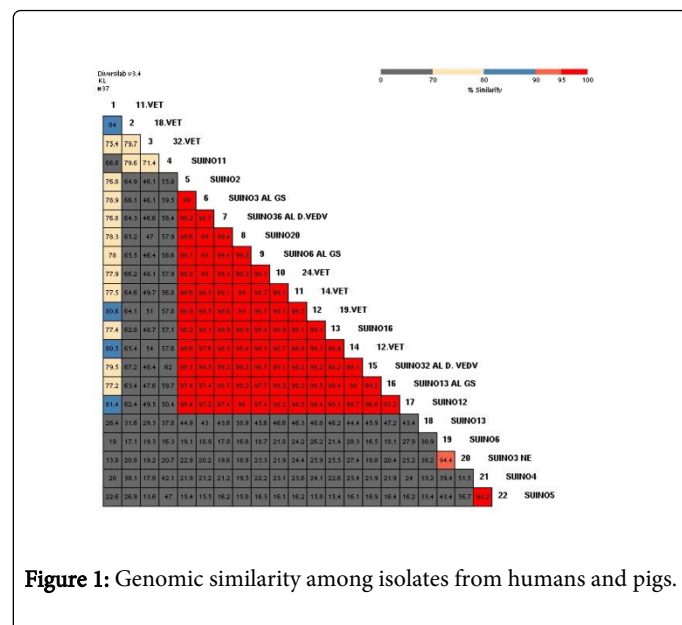


Figure 1: Genomic similarity among isolates from humans and pigs.

22 strains of MRSA were processed by rep-PCR. 7 were isolated by veterinarians and 15 from pigs. The analysis by DiversiLab placed them in 8 pattern, within which are distributed as shown in Table 2.

| Pattern N. | N. of isolates | Origin |
|------------|----------------|----------------|
| 1 | 1 | Vet |
| 2 | 1 | Vet |
| 3 | 1 | Vet |
| 4 | 1 | Swine |
| 5 | 13 | 4 Vet, 9 Swine |
| 6 | 1 | Swine |
| 7 | 2 | Swine |
| 8 | 2 | Swine |

Table 2: Breakdown of isolates among the genomic patterns

The pattern 5 is the one that includes more isolates, comprising 13 strains. In addition, it is also the only one in which they are represented MRSA both from the pig, both from man.

Discussion

As many as 13 of the 22 isolates subjected to genomic analysis were located in the pattern number 5, which is the only one including both strains derived from slaughter pigs and sows, and 4 veterinarians that are nasal carriers of MRSA.

Three of them are directly responsible for the management of large intensive pig farms of the Po Valley (located in Brescia, Mantua and Modena) while the fourth is full-time employed in an industrial pigs' slaughterhouse.

The common denominator of their carrier status is the continuous attendance of the different parts of the pig producing chain, which however require continuous contact with pigs.

The couples of isolates from pigs placed in patterns 7 and 08 come from farms geographically distant and from different farming system. This information is very interesting and could indicate a mechanism for the selection of bacterial clones, linked to more general characteristics of the system than to the management of each farm.

In the pattern 5 this indication is reinforced by the simultaneous presence of the isolates from veterinarians, which suggests the possibility that the selection of genomically similar MRSA takes place even in different geographical areas and that it is able to involve human occupationally exposed categories.

The results so far available allow confirming the hypothesis that motivated this research project, namely the existence of an epidemiological path of antibiotic resistance. The selection takes place in animals and occupational exposure related to specific risk factors is the gateway to the circulation in man.

Limitations

The potential limitations of this study are primarily related to the reduced number of vets still subject to verification of carrier status. However, other samples are already under way on other people at

occupational risk (farmers, slaughterers and butchers). On the isolates considered in this investigation the Spa-typing is also in progress to evaluate the prevalence of pathotypes in swine and veterinarians.

Conclusions

For further confirmation, to include other at-risk groups, such as farmers, their families and employees will be necessary, but also evaluating the contact time and its continuity. Equally important would be the assess of the risk of personnel involved in preparations to animals' feed, considering the risk of the contact with the antibiotic premixes but also considering timing and mode of survival of *S. aureus* in the workplace.

These early data suggest the possibility that MRSA represents the etiological agent of a new occupational zoonosis, in which the pig and its breeding systems can represent areas for the selection of antibiotic-resistance and therefore of pathogenicity of the bacterial strains.

The inter human diffusion of LA-MRSA derived from pig must also be investigated, also considering the spread to family members and life partners of workers in contact with pigs. The implementation of this option would give more importance to the need to control the use of antibiotics in pig production and therefore the role of veterinarians in achieving this goal.

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