MRSA transmission between pigs and veterinarians. A model of occupational risk.
De Faveri, E. (1), Fossati, P. (2), Castellani, I. (2), Sala, V. (1)*

Abstract
The populations of MRSA are classified in relation to their origin, differentiating Staphylococcus aureus Healthcare-Acquired (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 veterinarians employed in pig production (35.67% of nasal carriers). The fingerprinting comparison of man isolates with pig isolates indicated that many of the them belong to the same genomic patterns.

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 (Brumfitt & Hamilton-Miller, 1989). Only later, Methicillin-Resistant Staphylococcus Aureus (MRSA) were 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 (EARSS, 2008; NNIS, 2002) 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.

Materials and methods
157 veterinarians, working in different roles in pig production were included in the study. The sample included farm practitioners, operators and laboratorians in Veterinary Public Health, professionals working in pig industry and Commercial Companies and farm technicians. All samples were taken, prior informed consent, by unilateral nasal swab.

The sampling procedures were performed in compliance with Article 13 of the Italian Decree No. 106/2006, June 30 "Code on the protection of personal data". Each person has provided informed consent to the sampling procedure and personal details treatment. The project was previously approved by the Ethics Committee of the University of Milan. Nasal sampling was performed using disposable sterile swab with transport media (Ames W/OCH – Oxoid, Italy) Sample was aseptically performed in a non-traumatic way, avoiding any possibly contaminant contact. Swabs were immediately refrigerated (4-8°C) and transferred to the laboratory for analysis.

The swine strains came from the MRSA collection of the Swine Pathology Laboratory of the Veterinary Science and Public Health Department and were isolated from swine nasal swabs sampled in northern Italy.
pig-farms. The MRSA selective bacteriology was performed by the same laboratory, while the biomolecular
phase was achieved near the Microbiology, Virology and Bio-emergency Diagnostics Laboratory of the
"Luigi Sacco" University Hospital in Milan. The steps of the process are summarized below.

**Bacteriology of nasal swabs**
- Direct inoculation of MSA2 Gelose Chapman 2 Agar (Biomerieux, France) and of selective Oxacillin-
  Salt Screen Agar (Biolife Italiana). Both media were incubated at 37°C for 18-24 hours.
- Reading and interpretation. On MSA2 coagulase-positive staphylococci (\textit{S. aureus}) produce colonies
  with yellow halo, while coagulase-negative colonies are white and without color change. On Oxacillin
  Screen Agar, the strains able to grow can be considered oxacillin-resistant (the resistance is
  comprehensive of methicillin and nafcillin). The evaluation of growth on the two media allows the
  identification of methicillin-resistant \textit{S. aureus} (MRSA).
- After revitalization passage on 5% bovine blood agar, the isolates were frozen at -20°C in Nutrient
  Broth (Oxoid Italy) with 15% glycerol for one week and subsequently replicated on Tryptic Soy Agar
  (Biomerieux, France) before biomolecular survey.

**Molecular biology**

The methicillin-resistant strains of *Staphylococcus aureus* were analyzed by rep-PCR (DiversiLab System
- Biomerieux, France) near the Laboratory of Microbiology, Virology and Bioemergency Diagnostics of the
University Hospital Centre "Luigi Sacco" in Milan (Italy). Through this technique, similar isolates can be
classified in different genotypic patterns. Its application involves three subsequent steps: extraction of
bacterial DNA (via Ultraclean® Microbial DNA Isolation kit - Biomerieux, France), rep-PCR amplification
and detection of DNA fingerprinting by electropherogram. Through a dedicated software (DiversiLab -
Biomerieux, France) is therefore possible to compare the fingerprints and classify them in patterns. 42
isolates from man and 32 from pig were processed.

**Results and Discussion**

**Bacteriology**
The available results are organized in Table 1. They concern the isolations from nasal swabs of veterinarians
and technicians professionally involved in the pig industry. MRSA was widely isolated from nasal mucosa of
the employed in pig farming professionals, regardless of age and years of occupation. However, there is a
correspondence between the times spent in close contact with pigs, because the veterinary practitioners
are the core of positivity.

<table>
<thead>
<tr>
<th>n. of swabs</th>
<th>Negative</th>
<th>\textit{S. aureus} pos.</th>
<th>MRSA pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinarians n.</td>
<td>157</td>
<td>74</td>
<td>27</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>47.13</td>
<td>17.20</td>
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</tbody>
</table>

Table 1. Isolation of MRSA from Swine Veterinarians in Italy

**Molecular biology**
The results obtained by DiversiLab are organized in Table 2. 42 isolates from man and 32 from pig were
processed by rep-PCR. The applied system allows assessing the level of similarity of the genomic design of
isolates, expressing different patterns within which strains are genomically similar. Our analysis located the
isolates from man and pig in 8 patterns and the strains were distributed as shown in Table 2 and figure 1.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>N. isolates</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>vet</td>
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<td>2</td>
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<td>vet</td>
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<tr>
<td>3</td>
<td>2</td>
<td>vet</td>
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<tr>
<td>4</td>
<td>3</td>
<td>2 vet 1 swine</td>
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<tr>
<td>5</td>
<td>19</td>
<td>5 vet, 14 swine</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>29 vet, 13 swine</td>
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<tr>
<td>7</td>
<td>2</td>
<td>swine</td>
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<tr>
<td>8</td>
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Table 2. Breakdown of isolates among the genomic patterns.

**Figure 1.** Identification patterns. Graphical representation.

The pattern 5 and 6 were the most important, comprising 19 and 42 isolates respectively. Both showed
genomic similarities among isolates of the two species. Those of porcine origin prevailed in pattern 5, while
those of human origin were prevalent in pattern 6. Even pattern 4 comprised both species, but with only
three isolations. All other isolations included isolates coming from only one species.

**Conclusions**
The presence and circulation of MRSA in human communities and in intensive livestock farming are
proven and in many cases considered as unavoidable. The prevalence of silent localization is particularly
high and related to occupational exposure in pig farming.

In fact, of 157 veterinarians and farm operators, 56 (35.67% of the sample) were oro-nasal carriers of
MRSA. This percentage of positivity is greater than that reported in other exposed human categories and
pig-farms. The MRSA selective bacteriology was performed by the same laboratory, while the biomolecular phase was achieved near the Microbiology, Virology and Bio-emergency Diagnostics Laboratory of the “Luigi Sacco” University Hospital in Milan. The steps of the process are summarized below.

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The presence and circulation of MRSA in human communities and in intensive livestock farming are proven and in many cases considered as unavoidable. The prevalence of silent localization is particularly high and related to occupational exposure in pig farming.

In fact, of 157 veterinarians and farm operators, 56 (35.67% of the sample) were oro-nasal carriers of MRSA. This percentage of positivity is greater than that reported in other exposed human categories and
In addition, 36 isolates from humans (on 42 tested) were placed in the patterns 4, 5 and 6, together with isolates from pigs, while in the remaining 5 patterns, 10 strain of exclusive human or swine origin were found.

These results indicate the inter-specificity of MRSA clones. This feature enhances the diffusion and pathogenic capacity of any microorganism and methicillin-resistant S. aureus is particularly skilled in the expression of this ability.

We have already observed the MRSA spread to family members of vets, proving the epidemiological importance of the so-called Livestock-Acquired MRSA and their ability to reach the non-occupationally exposed population. The correlation between occupational exposure and biological risk is another aspect to be considered for MRSA, but also for other antibiotic-resistant bacteria. Moreover, the concept of biological risk must be revised, considering the progressive selection of bacterial clones in intensive pig farming.

Based on the available data, we cannot say that the risk is directly related to age or years of exposure. First of all, we should analyze the increase in the spread of the organism and from the increase of animal carriers. Our project finally considers the epidemiology of antibiotic-resistance path, along which the selection is made in the animal field and the occupational exposure can be the way entry into the circulation between people.

References

Abstract
Research over 10 years has established the high risk of occupational exposure to MRSA in people working with pigs. However, the nature of nasal colonization with livestock associated MRSA (LA-MRSA) and its health implications remain poorly understood. Multiple studies confirmed that detection rates in pigs, farm workers and veterinarians were higher than in people without animal exposure, but elevated risk of infection in these groups has not been clearly shown. We conducted a longitudinal study for 18 months to describe patterns of S.aureus (SA) colonization and infection in swine veterinarians. Overall, the monthly prevalence of SA and MRSA from nasal swabs of veterinarians was 58 to 82%, and 6 to 15%, respectively. Prominent spa types were t034 (ST398, 50%), t002 (ST5, 25%) and t037 (ST7, 18%), a distribution similar to that found in a concurrent study in pigs in the USA. Despite this high level of exposure, and frequent occurrence of minor injuries in the cohort, no confirmed cases of infection were reported. Based on detection patterns, veterinarians were classified into three groups: Persistent carrier (PC, 38%), Intermittent carrier (IC, 61%) and Non-carrier (NC, 1%). Based on one-time quantitative testing of nasal swabs without enrichment, PC veterinarians carried significantly higher numbers of SA than IC. Among IC veterinarians, culture positivity was significantly associated with recent contact with pigs. Elevated prevalence of SA and MRSA in US veterinarians appears to be a consequence of exposure to pigs, however MRSA prevalence was much lower than seen in a similar study in Holland. Exposure did not lead to prolonged colonization in most subjects, and the higher numbers of SA in PC subjects suggests that unknown host factors may determine the likelihood of prolonged colonization by SA of livestock origin. Although the period of follow up was limited, the absence of clinical infections despite regular exposure suggests that major health risks due to livestock associated SA are unlikely.

Introduction
Methicillin resistant Staphylococcus aureus (MRSA) was long perceived as a problem confined to the human medical arena, with animal reservoirs considered to be of negligible epidemiological significance. It is now more than a decade since the discovery of ST398 MRSA in pigs in the Netherlands undermined this paradigm. (Voss et al., 2005) In the interim, substantial research in many countries has consistently confirmed that people working in contact with live pigs (farmers, veterinarians, abattoir workers and their families) are at elevated risk of being culture positive for MRSA, due to exposure to livestock associated variants in the animal reservoir. While ST398 MRSA were initially referred to synonymously as ‘livestock associated MRSA’, the epidemiology of livestock associated MRSA is now known to be more complex. The predominant genotypes of MRSA in pigs have been found to vary geographically, with ST9 being the predominant lineage in Asia (Chuang and Huang, 2015). Previous studies of MRSA in pigs in North America have variably reported predominance of ST398 or ST5 variants, and ST9 variants have also been isolated. (Smith et al., 2009; Molla et al., 2012; Frana et al., 2013; Smith et al., 2013). Although isolates of the ST398 clonal complex have been predominant in most human studies, this likely reflects the status of the relevant swine populations. Individual studies have reported ST5 and ST9 isolates to be predominant in people exposed to pigs in North America and Asia respectively.(Neela et al., 2009; Frana et al., 2013)