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# Methicillin Resistant- and Methicillin Susceptible *Staphylococcus aureus*: Comparison of Genomic Similarity of Strains Isolated from Human and Veterinary Specimens Using Amplified Fragment Length Polymorphism (AFLP)

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**Abstract:** In consequence of a previous study on Methicillin-resistant (*MRSA*) and Methicillin-susceptible (*MSSA*) *Staphylococcus aureus* isolated from veterinary samples, a correlated study was carried out to evaluate the genetic relatedness among *MRSA* and *MSSA* isolated from human and veterinary specimens using the amplified fragment length polymorphism (AFLP) fingerprinting technique. Twenty-four out of 32 veterinary strains and 21 out of 29 human strains were *MRSA*. The methicillin resistance was evaluated with E-test and the presence of *mec A* gene was confirmed with PCR. The results of the genomic analysis revealed that all the isolated strains were distinct. An analysis of molecular variance (AMOVA) was also carried out to verify the distribution of variance a) among and within strains of different origin (human and veterinary) and b) among and within MRSA and MSSA strains. The results showed that in both cases the major component of variance was within strains (76.66 and 92.55%, respectively, for the first and second case).
A more accurate molecular technique, like AFLP rather PFGE, and the use of a sophisticate statistical analysis, like AMOVA, are strictly recommended to avoid that different strains are wrongly considered correlated.

**Key words:** Methicillin, resistance, methillin susceptible, polymorphism, genomic

## INTRODUCTION

*Staphylococcus aureus* is one of the most significant and widespread bacteria that causes infections of interest in both veterinary and human pathology[1-4]. The antibiotic resistant strains are widespread and most dangerous, and probably have been generated by an excessive use of antibiotics in the therapy of staphylococcal infections[5].

The methicillin-resistant *S. aureus* (*MRSA*) are of particular interest because they can cause further complications both in therapy and in epidemiology. The clinical-economic impact of these multiresistant and often lethal MRSA infections is a major public health problem[6].

MRSA in veterinary medicine are increasing; they have been isolated from bovine with mastitis, dogs and horses hospitalised[7-9] and have a zoonotic potential as demonstrated by Seguin[8].

In order to clarify the epidemiology and genetic relatedness of the *MRSA* and methicillin-susceptible *S. aureus* (*MSSA*) strains and eventually to identify those strains that are responsible for cross-infection and the spread of infections, an accurate fingerprinting technique, the Amplified Fragment Length Polymorphism (AFLP) analysis, was used.

The aim of this study was to determine if significant genetic differences were present between methicillin-resistant and methicillin-susceptible *S. aureus* isolated in the same area, and/or between strains coming from veterinary and human specimens.

## MATERIALS AND METHODS

Sixty-one *S. aureus* strains were used, 32 of which were isolated from veterinary specimens, dairy cows, sheep and dog and 29 from people hospitalised in different Units of Regional Hospital of Perugia, Italy.

Twenty-four out of 32 veterinary strains and 21 out of 29 human strains were *MRSA*. Samples cultured were submitted to methicillin resistance evaluation and AFLP technique and the results were statistically analysed as previously reported[10].

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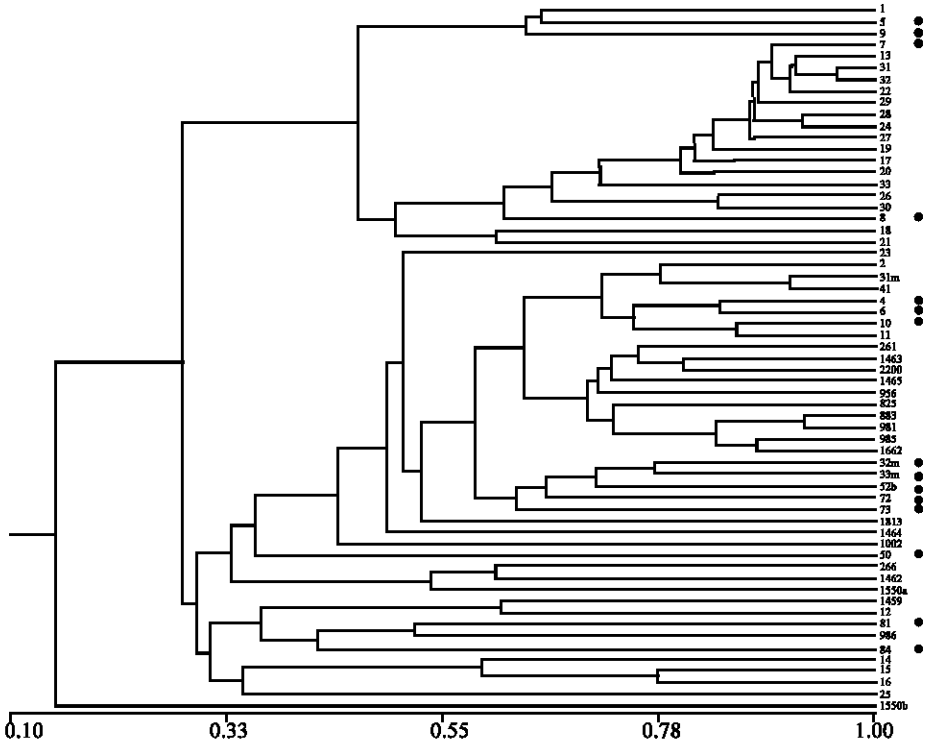


Fig. 1: Dendrogram of similarity. Cluster analysis of the Genetic Similarity data carried out following the UPGMA In bold (from n. 1 to n. 32) are indicated the

To obtain an optimum number of scorable and easily-comparable bands per primer combination, different numbers of selective nucleotides were tested on the samples. The best results were produced by a combination of two selective nucleotides in both *EcoRI* and *MseI* primers. The combinations of one and one or one and two selective nucleotides were discarded.

Two combinations, Eco+AC/Mse+GT (EM1) and Eco+AT/Mse+GG (EM2), were selected to analyse the 61 samples that produced 122 and 180 amplified fragments respectively, sized to within 1 bp and ranging in size from 100 to 500 bp. All bands of EM1 and EM2 were polymorphic.

The presence/absence data were used to generate a matrix of Genetic Similarities (GS) calculated by the Dice coefficient[11].

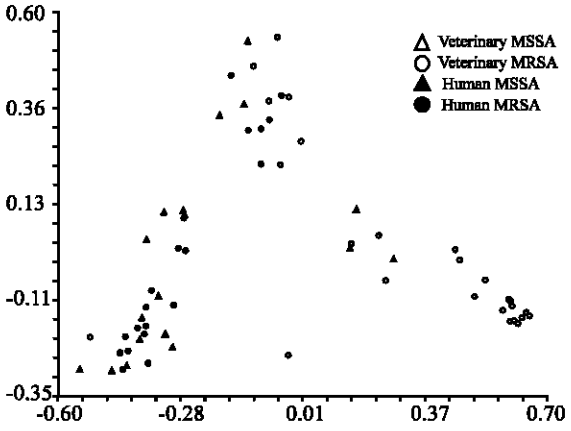


Fig. 2: Distribution of the samples produced by the Principal Coordinate Analysis (PCO).

## RESULTS

The average similarity was 0.3811±0.1819 for all samples, 0.4513±0.2221 for the veterinary samples and 0.4506±0.1755 for the human samples.

A cluster analysis of the GS data was carried out following the arithmetic average algorithm (UPGMA)[10] and the results were used to construct the dendrogram represented in (Fig. 1). As showed in the dendrogram, all the samples were distinct. The high cophenetic correlation

Table 1: a) AMOVA analysis among and within strains of different origin (human and veterinary);

| Source of variation | Degree of freedom | Sum of Squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among populations | 1 | 366.149 | 10.86159Va | 23.34 |
| Within populations | 59 | 2104.654 | 35.67210Vb | 76.66 |
| Total | 60 | 2470.803 | 46.5336900 | |
| Fixation index (FST) | | | 0.23341 | |

Significance tests (1023 permutations) Va and FST :
P(random value > observed value) = 0.00000
P(random value = observed value) = 0.00000
P(random value >= observed value) = 0.00000±0.00000

b) among and within MRSA and MSSA strains.

| Source of variation | Degree of freedom | Sum of Squares | Variance components | Percentage of variation |
|---|---|---|---|---|
| Among populations | 1 | 121.151 | 3.20471Va | 7.45 |
| Within populations | 59 | 2349.652 | 39.82462Vb | 92.55 |
| Total | 60 | 2470.803 | 43.0293300 | |
| Fixation index (FST) | | | 0.07448 | |

Significance tests (1023 permutations) Va and FST :
P(random value > observed value) = 0.00684
P(random value = observed value) = 0.00000
P(random value >= observed value) = 0.00684±0.00271

coefficient (r = 0.9404; P = 0.002) indicates strong agreement between the cluster diagram and the original similarity matrix. The Principal Coordinate Analysis (PCO)[10] produced a distribution of the samples (Fig. 2) that was similar to that of the dendrogram. In this analysis, the first two principal components accounted for 32.95% of the existing variation (24.10% for the first axis and 8.85% for the second).

The Analysis of Molecular Variance (AMOVA) [10] carried out to study the distribution of variance a) among and within strains of different origin (human and veterinary) and b) among and within MRSA and MSSA strains.

The results showed that in both cases the major component of variance was within strains (76.66% and 92.55%, respectively, for the first and second case) (Table 1).

The low level of similarity found between different *S. aureus* strains implies a high level of genetic diversity that was found in all strains independently of their origin. Results from this type of phylogenetic analysis become evident when the microrganisms studied are genetically very different. Although the strains isolated from animals were collected homogeneously in a limited time period and in the same environment, they nevertheless show a different genomic composition. The results do not specify if the genomic variation was correlated with the genes of virulence or of antibiotic resistance or with no-essential genes. As shown by the dendrogram and the 2D plot representation, there was a certain differentiation among human and veterinary strains. The same deduction could be obtained from the AMOVA analysis in which the value of among-strain variation was not very high (23.34%), but statistically significant. An earlier metabolic typing test failed to discriminate between isolates from different sources and suggested the utilization of other methods such as PFGE or AFLP[12]. The results of PFGE analysis placed the *S. aureus* isolated from human skin in the same pulsotypes as skin isolates from cows. On the other hand, no differentiation between the *MRSA* and the *MSSA* strains was found[12]. Similar results with a more completed and sophisticated analysis were obtained in other study[13]. For this study some strains were obtained from Haemodialysis Unit where the infection was related to the use of catheters. In patients with decreased resistance to infection, *S. aureus* is a major cause of bacteraemia and its complications[14]. The same way of infection could be observed in dairy cows when the antibiotic is inoculate directly in the teats. To prevent the colonization of these plastic materials the pharmaceutical industries are studying the possibility of adsorbing the antibiotics onto different polymers as a possible experimental model for reducing the incidence of infection by *Staphylococcus* and other organisms[15].

## CONCLUSIONS

This study put in evidence the necessity to use a more accurate molecular technique like AFLP rather PFGE when a comparison of different strains is carried out. Furthermore, the use of a sophisticate statistical analysis, like AMOVA, is strictly recommended to avoid that different strains are wrongly considered correlated.

## REFERENCES

1. Devriese, L.A., 1975. Epidemiology of methicillin-resistant *Staphylococcus_aureus* in dairy herds. Res. Vet. Sci., 19: 23-27.
2. Scott, G.M., R. Thompson, J. Malone-Lee, G.L. Ridgeway, 1988. Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in human. J. Hosp. Infect., 12: 29-34.
3. Emmerson, M., 1994. Nosocomial staphylococcal outbreaks. Scand. J. Infect .Dis., 93: 47-54.
4. Rezende, N.A, H.M. Blumberg, B.S. Metzger, N.M. Larsen, S.M. Ray, J.E. Mc Gowan, Jr., 2002. Risk factors for methicillin-resistance among patients with Staphylococcus aureus bacteraemia at the time of hospital admission. Am. J. Med. Sci., 323: 117-123.
5. Pérez-Roth, E., F. Claverie-Martin, J. Villar, S. Méndez-Alvarez, 2001. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J. Clin. Microbiol., 39: 4037-4041.
6. Agodi, A., F. Campanile, G. Basile, F. Viglianisi, S. Stefani, 1999. Phylogenetic analysis of macrorestriction fragments as a measure of genetic relatedness in *Staphylococcus aureus*: the epidemiological impact of methicillin resistance, Eur. J. Epidemiol .,15: 637-642.

7. Cuteri, V., R. Mazzolla, F. Valente, L. Merletti, C. Valente,. 2002. Applicazione della elettroforesi pulsata (PFGE) a stipiti meticillino-resistenti di *Staphylococcus aureus* isolati dall'uomo e dagli animali. Le infezioni in medicina 1: 25-30.
8. Seguin, J.C., R.D. Walker, J.P. Caron, W.E. Kloos, C.G. George, R.J. Hollis, R.N. Jones, M.A. Pfaller, 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission, J. Clin. Microbiol., 37: 1459-1463.
9. Pak, S.I., H.R. Han, A. Shimizu, 1999. Characterization of methicillin-resistant *Staphylococcus aureus* isolated from dogs in Korea. J. Vet. Med. Sci., 61: 1013-1018.
10. Cuteri, V., M.L. Marenzoni, R. Mazzolla, N. Tosti, L. Merletti, S. Arcioni, C. Valente, 2004. Staphylococcus aureus: Study of genomic similarità of strains isolated in veterinary pathology using amplified fragment length polymorphism (AFLP). Comp. Immun. Microbiol. Infect. Dis., 27: 247-253.
11. Dice, L.R., 1945. Measures of the amount of ecological association between species. Ecology 26: 297-302.
12. Cuteri, V., R. Mazzolla, L. Merletti, C. Valente, 2001. *Staphylococcus aureus*: application of pulsed field gel electrophoresis (PFGE) to bovine methicillin-resistant strains. Giorn It Mal Inf ., 7: 1-3.
13. Fitzgerald, J.R., D.E. Sturdevant, S.M. Mackie, S.R. Gill, J.M. Musser, 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. In: Proceeding of National Academy of Science, USA: National Academy of Science, 98: 8821-8826.
14. Shinefield, H., S. Black, A. Fattom, G. Horwith, S. Rasgon, J. Ordonez, H. Yeoh, D. Law, J.B. Robbins, R. Schneerson, L. Muenz, S. Fuller, J. Johnson, B. Fireman, H. Alcorn, R. Naso, 2002. Use of a *Staphylococcus aureus* conjugate vaccine in patient receiving haemodialysis. N. Engl. J. Med., 346: 491-496.
15. Maki, D.G., S.M. Stolz, S.J. Wheeler, L.A.Mermel, 1997. Prevention of central venous catheter related bloodstream infection by use of an antiseptic-impregnated catheter. Ann. Intl. Med.,27:257-266.