

## ARTICLE / INVESTIGACIÓN

## Identification of methicillin-resistant strains of *Staphylococcus aureus* isolated from humans and food sources by use *mecA 1* and *mecA 2* genes in Pulsed-field gel electrophoresis technique

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**Abstract:** *Staphylococcus aureus* causes mastitis in dairy cows, lambs, goats, and skin disorders in pigs and other animals. *S. aureus* causes localized purulent infections that affect soft tissues, bones, and other organs in humans. Using restriction patterns, the researchers want to isolate and identify methicillin-resistant *Staphylococcus aureus* (MRSA) strains from cattle and humans. They also hope to assess their genetic relatedness by comparing the *mecA1* and *mecA2* gene sequence discrepancies. Animals (223 strains) and people have been used to acquire *S. aureus* strains for study (83). The E-test was used to assess whether or not the bacteria were resistant to methicillin. The *mecA1* and *mecA2* genes were identified by using pulsed-field gel electrophoresis (PFGE) to analyze DNA restriction patterns. The results were shown. *S. aureus* strains from animals and men were resistant to methicillin in 32 (14.34 %) and 53 (63.8 %), respectively. PFGE was used to determine the differences between human and veterinary pathology strains. Two strains of bacteria collected from animals were discovered to be identical; nevertheless, microorganisms recovered from humans were found to be significantly similar to the bacteria recovered from animals. Both human and veterinary pathology were implicated in the development of methicillin resistance. The MRSA strains found in humans were much more significant than those found in animals. The strains recovered from animals exhibited a high degree of genetic heterogeneity. Still, the enormous number of indistinguishable bacteria in humans leads one to believe that a dominant clone is present. When it comes to the molecular characterization of MRSA isolates, PFGE might be regarded as the gold standard.

**Key words:** Animals, Human, MRSA, PFGE, *Staphylococcus*, *mecA* genes.

### Introduction

A report on the occurrence of methicillin-resistant *S. aureus* (MRSA) in medicine was published soon after the antibiotic methicillin was introduced into clinical practice to treat infections caused by these germs<sup>1</sup>. Characteristics of staphylococci that increase their pathogenicity include their propensity to colonize and multiply actively. When the infection takes hold, contributing risk factors include the slow healing of certain recurring infections, the use of lengthy antibiotic treatments, the use of catheters, and, once again, the presence of open wounds<sup>2</sup>. In dairy cows, *S. aureus* is one of the most common agents responsible for mastitis<sup>3,4</sup>, sheep and goats and is often involved in skin infections in pigs and other animal species<sup>5</sup>. *S. aureus* is the leading etiological agent of localized purulent infections affecting soft tissues, bones, and other organs in humans. It is mainly responsible for infections of natural exogenous according to its ability to spread in the environment, thus causing epidemics in closed communities such as hospitals, homes in care and schools<sup>6-8</sup>. Under some situations, the microbe demonstra-

tes a high virulence capability, capable of exacerbating the inflammatory process till the host dies. Methicillin-resistant strains have been recorded in dogs<sup>9</sup>, horses, and dairy cows after treating staphylococcal infections with methicillin and -lactams in general<sup>4</sup>. In humans, the MRSA strains are fearsome pathogens, especially in the hospital field, and countless epidemics are reported in the bibliography<sup>7</sup>. It has recently been proven that the transmission routes can be mainly two, one direct from patient to patient and the other, indirect, through healthcare professionals<sup>7,10</sup>. Methicillin resistance arises due to the presence of the *mecA* genes those code for a Penicillin Binding Protein (PBP2a), which has a low affinity for  $\beta$ -lactam compounds<sup>11</sup>. Veterinary pathology has long recognized that resistance to methicillin and oxacillin, a closely related antibiotic extensively used in veterinary pathology, is often linked with analogous behavior against a broad spectrum of antibiotics<sup>12</sup> to compromise therapeutic interventions, which, concerning bovine mastitis, represent the most effective method for controlling

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the disease. Now the 1960s, MRSA isolates in people have been recorded in European hospitals, and the disease has since expanded to non-hospital settings as well<sup>13</sup> so much so that, At the moment, MRSA jams account for 25 percent of the strains of *S. aureus* that cause nosocomial infections in hospitals. In this work, the goal was to determine the existence of isolated MRSA strains in both human and animal samples and submit these jams for molecular examination of chromosomal DNA through PFGE.

## Materials and methods

### Bacterial isolates

From the mammary secretion of cows with mastitis subclinical, 223 *S. aureus* isolates have been obtained. Of patients admitted to different wards of the hospital, 83 were selected from the isolates of *S. aureus*. The pathological material was sown in Nutrient Agar with the addition of 5% sheep blood and in Mannitol Salt Agar. The isolated microorganisms were identified by coagulase and Dnase tests, API Staph.

### Methicillin-resistance

Mueller Hinton Agar was used to cultivate the isolates. Following that, 4-5 isolated colonies were collected and planted in Nutrient Broth up to a turbidity of 0.5 McFarland in 2-8 hours at 37°C till the turbidity of 0.5 McFarland was reached. For the determination of the Minimum Inhibiting Concentration (MIC) expressed in micrograms per milliliter (g/ml), an aliquot of 0.01 ml was diluted in 10 ml of saline solution, and 3 ml were distributed on the surface of a plate Mueller Hinton Agar<sup>14</sup> on which they have placed the nitrocellulose strips containing Methicillin E-test for the determination of the Minimum Inhibiting Concentration. Following incubation at 35°C for 24 hours, *S. aureus* jams were tested for resistance (MRSA). Those that showed an equal or more significant inhibition at µg / ml were designated as resistant (MRSA). As a result of PCR<sup>15</sup>, the existence of the *mecA* gene has been identified. The QIAmp tissue Kit was used to perform the DNA purification. The primers *mecA1* (5'-AAA ATC GAT GGT AAA GGTTGG C) and *mecA2* (5'-AGT TCT GCA GTA CCGGAT TTG C) highlight a product of PCR of 533bp<sup>16</sup>. Ungeheuer's method of DNA amplification was used<sup>17</sup>. DNA electrophoresis was carried out on a gel containing 2 % agarose, after which it was colored with ethidium bromide and photographed under the influence of ultraviolet light.

### Pulsed-field gel electrophoresis (PFGE)

The macro-restriction approach with Sma I described the MRSA jams, which were then resolved by pulsed electrophoresis<sup>18</sup>. In 10 ml of Trypticase Soy Broth at 37°C for

24 hours, the bacteria were grown before being centrifuged at 2000 g for 10 minutes, and the pellet was resuspended in PIV buffer. To manufacture the plugs, an aliquot of 0.5 ml of the latter suspension was combined with an equal quantity of Incert Agarose (low melting) 1 % to form a homogeneous mixture. Each plug received one milliliter of lysis solution, and they were incubated at 37°C for 24 hours in agitation. The lysis solution was replaced with a protein digestion buffer and then incubated at 50°C for 48 hours under agitation. The plugs were washed with TE and PMSF and three more times with TE. Later iplugs were subjected to enzymatic digestion in a mixture of H2O sterile, spermidine (1 mM), Smal 100U. After boiling for around 24 hours at 25°C in a final volume of 250 mL, the solution was utilized for pulsed electrophoresis on a 1 percent Fast Lane agarose gel at 14°C in TBE buffer auto-algorithm for 24 hours. Lambda Ladder was the standard that was utilized. The PFGE was done out using the CHEF Mapper system, which may be found here. To build a similarity dendrogram<sup>18</sup>, the patterns electrophoretic were captured using a camera in the Molecular program Analyst Finger Printing and analyzed using the MA Finger Printing. The similarity coefficients (SAB) were estimated using software that was not modified in any way. From 0 to 1, the SAB indicated whether or not the patterns were connected, with 0 indicating that they were not related and one suggesting that they were tightly associated.

## Results

The chemical and metabolic properties of the 306 *S. aureus* isolates were consistent throughout the group. Of the 223 jams studied, 32 (14.34 percent) were found to be resistant to methicillin, but of the 83 jams investigated that were of human origin, 53 (63.8 percent) were found to be MRSA. Forty MRSA and twenty MSSA jams, derived in equal parts from human and veterinary illness, in which the presence of the *mecA* gene has been validated by PCR to verify the findings obtained with the E-test and Sceptor System, have been subjected to PFGE. As a consequence of the PFGE data, the investigation of the genetic similarity of the MRSA jams, which was shown using a dendrogram (Figs. 1 and 2), revealed that the strains of human and veterinary origin were distinct. The blocks that arrived showed a significant degree of resemblance among themselves; however, just two strains of animal origin showed identical results compared to the unions that came (Table 1).

## Discussion

*Staphylococcus aureus* is one of the most prevalent germs found in nature and one of the most responsible for illness, both in people and in animals. It can produce infections that are difficult to treat with conventional therapy<sup>19</sup>.

Resource	Indistinguishable	Closely related	Probably related	Not related	Total
MRSA Human isolates	6	6	6	2	20
Animal isolates	2	2	0	16	20
MSSA Human isolates	2	3	2	3	10
Animal isolates	0	2	2	6	10

**Table 1.** *Staphylococcus aureus*: genomic similarity of the isolates from human and animal pathology.

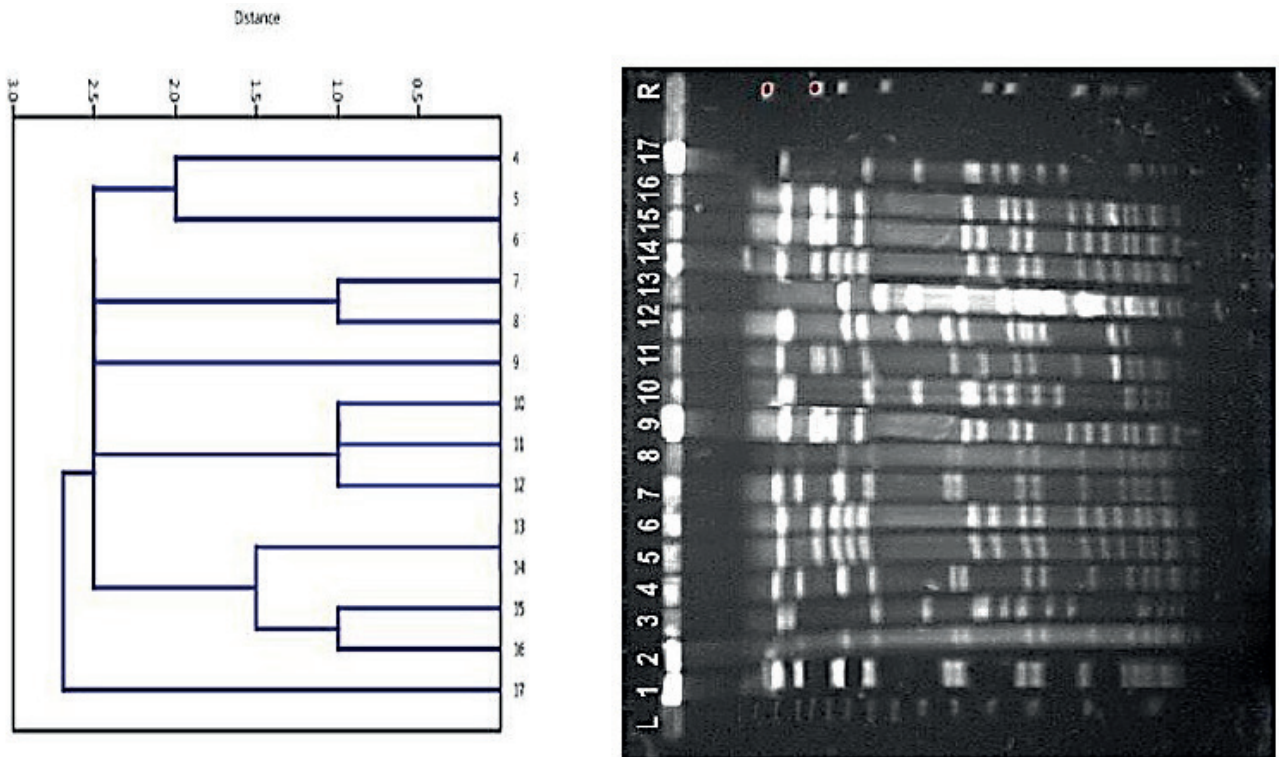


Figure 1. *Staphylococcus aureus* (MRSA) of human origin: similarity dendrogram.

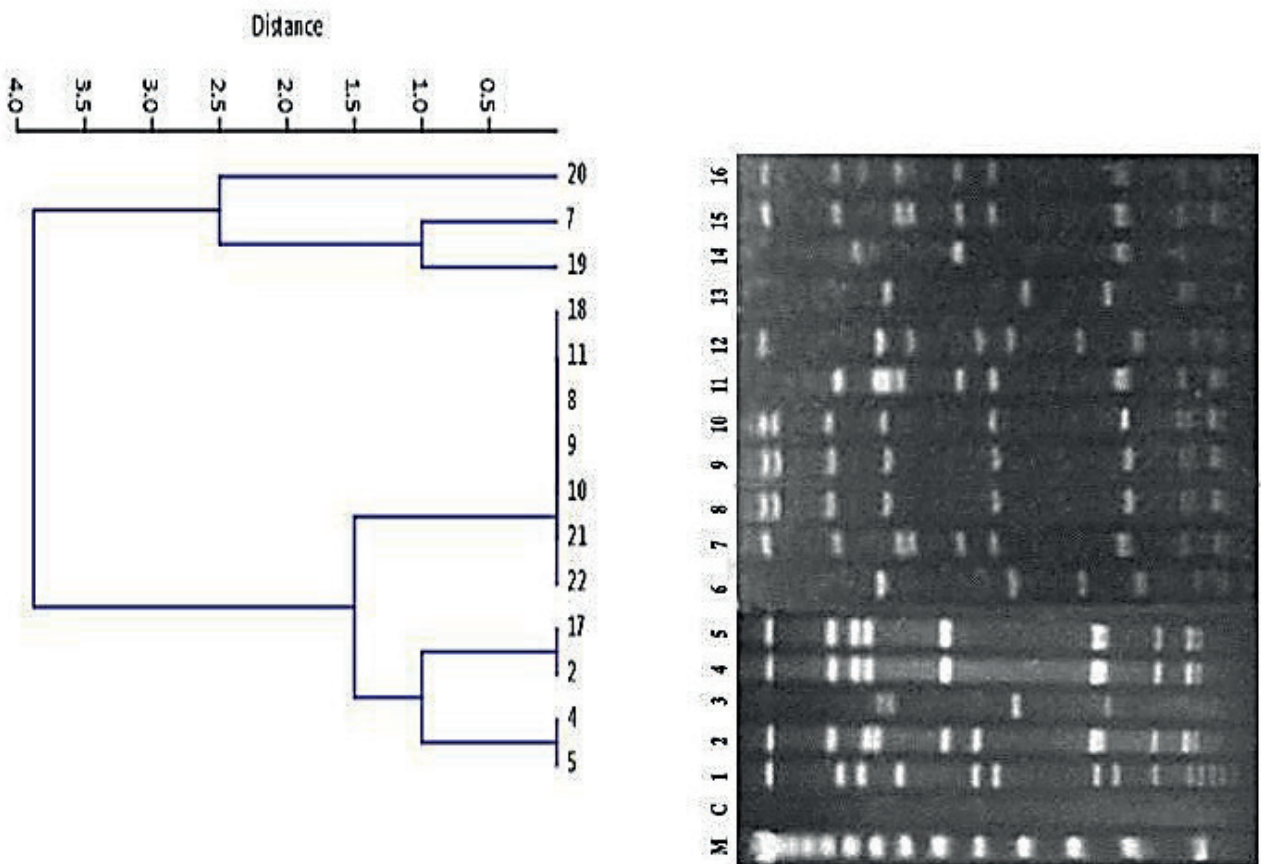


Figure 2. *Staphylococcus aureus* (MRSA) of animal origin: similarity dendrogram.



To adapt to the increased use of antibiotics, *S. aureus* has modified its genomic structure and made the PBP2a protein via the *mecA* genes, which has a low affinity for  $\beta$ -lactam antibiotics, leading in the appearance of methicillin-resistant jamps<sup>18,20</sup>. One of the issues that must be addressed is selecting a technique for identifying staphylococcal jamps. The identification of MRSA strains is difficult, even with the international set of phages, and it was therefore deemed appropriate to replace conventional methods with molecular biology techniques, such as PFGE or polymerase chain reaction, which, when performed with the primers *mecA1* and *mecA2*, results in the identification of a sequence of 533 base pairs, specific for MRSA jamps<sup>14</sup>. Since it has been demonstrated in human pathology that MRSA jamps from different continents are classified as indistinguishable, it was deemed appropriate to compare jamps of human and veterinary origin, in the same territorial context, to verify if they had been realized under the same conditions. Although the jamps of animal origin originated from farms in different geographies, this does not excuse the lack of genetic similarity between them, as MRSA jamps considered unrecognizable have a genetic similarity of 80% or greater<sup>21,22</sup>. When a comparison analysis was conducted between the 40 MRSA jamps and the 20 MSSA jamps, it was discovered that there is no genetic similarity between them, just as there were no commonalities between the MRSA jamps from veterinary and human pathology. However, only two MRSA jamps of veterinary origin could be identified as genetically identical and two other closely related ones.

In contrast, in human pathology, the genetically similar jamps (n.6) and closely related jamps (n. 6) suggested the presence of a dominant strain in the degree to which there is a close correlation between strains isolated over time. Aside from the genetic similarities, the difficulties associated with methicillin resistance impact veterinary and human illnesses similarly. Pulsed electrophoresis is widely recognized as the gold standard for molecular typing of a broad variety of microorganisms. It has been shown to be highly selective and equivalent, if not better, to other approaches for a large number of harmful bacteria<sup>4,16,18</sup>. The findings of the investigation, despite being constrained by a small number of jamps, confirm the presence of MRSA in cattle and demonstrate the high level of genomic variability found within methicillin-resistant strains, which suggests that these bacteria could spread from animals to the environment through contact with other animals other than humans<sup>20</sup>. As an additional indication of the complex somatic structure of *S. aureus*, there is an inverse relationship between the homogeneity shown by chemical-metabolic analysis and genomic inequality, which is the element that best defines the bacterium.

## Conclusions

MRSA isolates from several hospital wards have been studied. The results indicate a significant degree of similarity between certain jamps, which suggests the presence of a typical clone from which they might originate the isolates. This clone has been present at the hospital for at least 15 years. It has caused periodic epidemics (data not published), leading some to speculate that it has colonized the healthcare workers, who would then act from tanks and vehicles due to the outbreak. The genetic analysis of MRSA strains highlights the necessity for adequate procedures to

be taken to monitor clinical isolates so that they may act effectively to stop the epidemiological cycle, as shown by the research.

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## Conflicts of Interest

No conflict.

## Funds

Self by authors

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