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ISOLATION AND CHARACTERIZATION OF SOME METHICILLIN RESISTANT *STAPHYLOCOCCUS SPP.* STRAINS ISOLATED FROM MASTITIC BOVINES

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Summary

In recent years, a special attention is paid to methicillin-resistant staphylococci strains, which have a pronounced risk and a complex epidemiological circuit, being common in humans, as well. The research was made on 20 samples of mastitic milk taken from primiparous bovines from a cattle breeding farm in Timiș county. From the taken samples, 64 strains belonging to *Staphylococcus* genus were identified and included in 19 species of staphylococci. These species isolated on selective media and definitively identified with the Vitek 2 Compact equipment were tested for resistance to methicillin. Thus, three antibiotics, namely methicillin, oxacillin and cefoxitin, were used, of which the highest resistance frequency wasto methicillin.

Keywords: methicillin, resistant, staphylococci, strains

Starting from the numerous studies conducted at national and international level to demonstrate the extent of multiple resistance to antimicrobial substances phenomenon present at various bacterial species, we consider that such research is necessary because it will generate a valuable set of data from epidemiologically point of view (2, 5, 10, 12).

Methicillin-resistant staphylococci are considered to be zoonotic risk bacteria because methicillin resistance is associated with multiple antibiotic resistance. Methicillin-resistant strains are monitored both in human and veterinary medicine and the study of the animal-human-animal circuit of these strains is a major public health concern (1, 2, 3, 7).

In cattle, staphylococcal infections are common and are represented by clinical and subclinical mastitis, laminitis or other localized infections. Mastitis are produced by both positive and negative coagulase staphylococci, with a correlation between the progressive clinical forms and the pathogenicity of the strains (5).

The research was performed in order to identify the resistance phenotypes of staphylococci strains isolated from primiparous bovines with mastitis.

Materials and methods

Pathological samples, represented by mastitic milk, were taken from primiparous cows that presented mastitis. Primary sowings were performed on

agar with defibrinated blood of 5% sheepand isolated strains were screened based on cultural, morphological and tinctorial characters. To obtain pure staphylococci cultures, the solid Chapman medium was used, while the final identification was made with the Vitek 2 Compact equipment, using identification cards for enterococci, streptococci, staphylococci, and a select group of Gram-positive organisms. Thus, of the 20 collected samples, several strains belonging to the *Staphylococcus* genus and included in different species of staphylococci were identified.

The isolated strains were tested for methicillin resistance by Kirby-Bauer disc-diffusion method using broth, Mueller-Hinton agar and three antibiotics, namely methicillin (5 µg), oxacillin (1 µg) and cefoxitin (30 µg) (Table 1), the results being interpreted according to the standards (6).

Results and discussions

The bacteriological examination, carried out according to thedescribed methodology, followed by definitive identification with the Vitek 2 Compact equipment, allowed the isolation of 64 strains, which were included in 19 species of staphylococci.

The results obtained by testing the behavior against the three β-lactams of the staphylococcal isolated strains are shown in Table 1.

Table 1

Resistance phenotypes to the three antibiotics of staphylococci strains isolated from mastitic cows

Crt. no.	Antibiotic	Antibiogram results						Total strains
		Susceptible		Intermediar		Resistant		
		No.	%	No.	%	No.	%	
1.	Cefoxitin	55	85.93	4	6.25	**5**	**7.81**	64
2.	Oxacillin	20	31.25	8	12.5	**36**	**56.25**	64
3.	Methicilin	18	28.12	9	14.06	**37**	**57.81**	64

The results show that the antibiotic resistance of the tested strains had a variable frequency ranging from 7.81% to 57.81%, the antibiotic susceptibility was between 28.12% and 85.93%, and the strains with intermediate behavior had had a frequency between 6.25% and 14.06%.

These three antibiotics used, namely methicillin, oxacillin and cefoxitin, are part of the β-lactam group and have been selected because they are antibiotics that show methicillin resistance. Oxacillin is resistant to β-lactamases, being preferred for the stability and reproducibility of the results, and since 2004, cefoxitinis also recommended, especially for the identification of *S. aureus* methicillin resistant strains.

Isolated staphylococci strains had a different behavior to **methicillin**, a commonly used antibiotic for the detection of methicillin-resistance. Thus, 57.81% of the tested strains were resistant to this antibiotic, 28.12% of the tested strains were susceptible and 14.06% had an intermediate resistance.

To determine the cross-resistance of staphylococci to the penicillins resistant to penicillinase, oxacillin is also recommended for both its stability and the reproducibility of the results. The results obtained using the oxacillin were as follows: 56.25% were resistant strains, 31.25% were susceptible strains and 12.5% had an intermediate behavior.

The increased frequency of methicillin-resistant staphylococci strains and, in particular, strains of *S. aureus subsp. aureus*, named MRSA strains (Methicillin Resistant *S. aureus*), also determined testing the resistance to cefoxitin, a much more reliable antibiotic than methicillin and oxacillin. The results obtained by testing the isolated staphylococci strains to cefoxitin were as follows: 7.81% of the strains were resistant, 85.93% of the strains were susceptible and 6.25% of the strains had an intermediate behavior (Fig. 1).

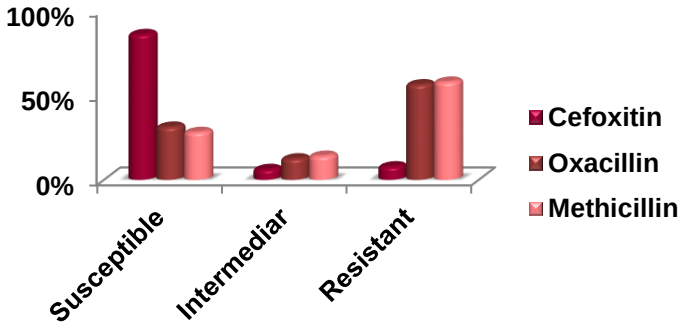

Fig.1. Frequency of resistance phenotypes of the isolated staphylococci strains

The antibiotic resistance to these three β-lactams was different, observing a correlation only between oxacillinand methicillin, whereas only 7.81% of the tested strains were resistant to cefoxitin.

In the tested staphylococci strains, the resistance to methicillin was 57.81%, demonstrating a high proportion of the strains carrying the *mec* gene encoding the resistance to this antibiotic.

Methicillin-resistant staphylococci strains are considered strains with high zoonotic risk and with a complex epidemiological circuit. The *mec* gene encoding the methicillin resistance may be transmitted by the R plasmid to methicillin-susceptible staphylococci strains (intraspecific transmission) strains, but also to

other strains of other bacterial species (interspecific transmission).The results obtained show the correlation between oxacillin and methicillin resistance, but do not prove the correlation between the resistance of these two β-lactams and cefoxitin, of which the antibiotic resistance was very low, phenomenon also reported in the literature (1, 2, 4).

The resistance phenotype to methicillin is commonly found in strains of *S. aureus subsp. aureus*, isolated from humans and animals, strains called MRSA. In recent years, however, this phenotype has been reported more commonly in other staphylococcal species pathogenic to humans and animals (4, 13).

In Brazil, in 2012, Aquino et al. (3) followed prevalence of methicillin resistant staphylococci strains to several species of animals, by taking samples of pathological material from cow milk (36), cattle meat (26), sheep (19), horse (21), pig (23), goats (23) and care staff (13). The authors isolated 161 staphylococcis trains, at which they study the antibiotic resistance against 17 antibiotics, and the *mec* gene was detected with the polymerase chain reaction. Coagulase-negative staphylococci, resistantto methicillin, had a frequency of 31%. The authors did not isolate strains of *S. aureus* but identified a complex epidemiological circuit between animals and humans of methicillin resistant coagulase-negative strains (3).

Mendonça et al. (9) in 2012, studied the resistance patterns of *Staphylococcus spp.* strains isolated from cattle by phenotypic tests (disc diffusion method) and molecular biology. The authors recommend oxacillin to determine the frequency of *Staphylococcus spp.* methicillin resistant strains, considering this method as a standard method, as well as the PCR test for the detection of the *mec*A, *mec*I and *mec*RI genes and the *bla*Z gene.Based on the results obtained, the cited authors found a close correlation between the disk diffusion method with oxacillin and the presence of the mentioned genes (9).

In 2012, in Germany, Fessler et al. (7) have studied the prevalence of MRSA strains isolated from animals and humans. Following the bacteriological examination, 125 MRSA strains were isolated as follows: 46 strains from milk samples, 24 strains from dairy cows, 7 strains from calf, 16 strains from pigs, two strains from dogs and 28 strains from horses, sheep and humans. All strains were included in the CC398 gene line, and the SCC*mec* 4 cassette was detected by the polymerase chain reaction for 48 strains and the SCC*mec* type 5 cassette for 67 strains. Based on the results, the authors believe that there is a complex epidemiological circuit of the MRSA strains among the investigated animal species and humans that can generate pronounced zoonotic risk infections (7).

Bardiau et al. (4), in 2013, in Belgium, studied the frequency of methicillin-resistant strains at a number of 430 staphylococci strains isolated of from cows with clinical and subclinical mastitis. Phenotypic and genotypic tests performed allowed the identification of 19 strains of *S. aureus subsp. aureus* methicillin-resistant. Also, the ST398LA-MRSA clone, considered emerging and with zoonotic risk, was identified by the PCR technique and the pulse field macro-restriction technique (4).

Schlotter et al. (11), in 2013, bacteriologically examined 10421 cattle from 34 farms, being able to isolate 1902 strains of *S. aureus subsp. aureus*. These strains were tested for antibiotic resistance by disc-diffusion method and were tested by PCR for the detection of the genes responsible for coding this phenomenon. A number of 135 strains did not have resistance genes, and at the other strains the *mec*A, *erm*A, *erm*B, *erm*C and *msr*A genes had a variable frequency. The authors believe that *mec*A gene detection is necessary to identify the methicillin-resistant *S. aureus subsp. aureus* strains (11).

In 2017, Mello et al. (8) studied the frequency of resistance to oxacillin and vancomycin in a number of 181 strains of *Staphylococcus spp.*, isolated from subclinical mastitis, as well as the identification of heteroresistance to vancomycin by a screeching method. The authors also identified the *mec*A and *mec*C genes. The results were as follows: 18.2% of the strains were oxacillin-resistant, all isolated strains were vancomycin-susceptible, while the heteroresistance was observed in 13 strains and the *mec*A gene was identified in 8 strains, all included in *S. epidermidis* species. Based on the results obtained, the authors state that it is necessary to evaluate the strains isolated from cows with mastitis from bacteriologically and molecularly point of view, since the presence of *mec*A gene in *S. epidermidis* demonstrates that the cow's milk can be a carrier of resistant strains of human origin, highlighting the epidemiological circuit of these strains (8).

The results obtained confirm the data in the literature on the frequency of resistance patterns of the three resistant beta-lactams and, in particular, the frequency and zoonotic risk of MRSA strains.

Conclusions

The results obtained on resistance phenotypes to three beta-lactams, resistant to penicillinase, have revealed a different frequency of the strains resistant to these three antibiotics.

Testing for resistance to oxacillin detected a higher frequency compared to methicillin resistance testing.

Testing for cefoxitin by the disc-diffusion method revealed a frequency of 7.81% of resistant strains, strains that can be considered MRSA strains.

At the isolated strains, the resistance to methicillin and oxacillin was similar, revealing a high frequency of strains carrying the *mec* gene.

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