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THE USE OF A CHROMOGENIC MEDIUM FOR THE IDENTIFICATION OF METHICILLIN-RESISTANT STAPHYLOCOCCI

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Abstract

In the last years, an increasing attention is paid to methicillin-resistant staphylococci, isolated from animals, regardless of the species they are included in. The circulation of methicillin-resistant staphylococci strains is monitored by phenotypic laboratory techniques or with several chromogenic media. The frequency of methicillin-resistant strains was pursued on 412 strains included in S. aureus subsp. aureus and other species from the "non-S. aureus" group, based on phenotypic characters. Using the disc-diffusion method with methicillin, oxacillin and cefoxitin, 210 strains resistant to methicillin were identified and poured into a chromogenic medium named Chromatic™ MRSA. On this medium, S. aureus subsp. aureus strains resistant to at least one of the three antibiotics, formed purple to purple-orange colonies and the "non-S. aureus" strains formed white or blue colonies. All S. aureus subsp. aureus strains on this medium were methicillin-resistant, results identical to the disc-diffusion method ones. 146 "non-S. aureus" strains formed white or blue colonies, considered as methicillin-resistant, while 6 strains did not grow on this medium.

Key words: *chromogenic medium, methicillin-resistant, staphylococci.*

INTRODUCTION

Resistance to methicillin, in coagulase-positive and coagulase-negative staphylococci isolated from farm animals and pets, is very topical, which is why many researchers are studying this phenomenon in many countries. Given the large number of staphylococci species isolated from animals, which cause systemic or localized infections through pathogenicity factors (enzymes, exotoxins, biofilm), most researchers, in antibioresistance and methicillin-resistance research, use either the staphylococci divided into two groups, namely coagulase-positive and coagulase-negative staphylococci or the term "non-*S. aureus*" for all species of staphylococci except *S. aureus subsp. aureus* (Fowoyo P. T. et al., 2017; Kunz F. et al., 2011; Loeffler A. et al., 2013; Park J. et al., 2013; Saputra S. et al., 2017).

In recent years, an increasing attention has been paid to positive and negative coagulase staphylococci strains, resistant to methicillin, regardless of the species they are included in. Circulation of methicillin-resistant strains is monitored by phenotypic laboratory techniques, namely the Kirby-Bauer disk-diffusion method, the chromogenic medium method and molecular biology techniques (Dupieux C. et al., 2017; Saito E. et al., 2011).

In the screening studies conducted to monitor the circulation of methicillin-resistant strains, cromogenic media that selectively act to differentiate strains of *S. aureus subsp. aureus* versus „non-*S. aureus*" staphylococci strains are commonly used. This differentiation is based on the color of staphylococcal colonies (Saito E. et al., 2011).

The research aimed the frequency of methicillin-resistant strains, isolated from several animal species, using the Chromatic™ MRSA medium.

MATERIALS AND METHODS

The frequency of methicillin-resistant strains was followed in 412 staphylococci strains included in 22 species. Initially, the frequency of these strains was determined by the Kirby-Bauer disk diffusion method, using biodiscs with methicillin (5 μg), oxacillin (1 μg) and cefoxitin (30 μg). The methicillin-resistant strains detected by this method were the tested using the Chromatic™ MRSA medium, which is used to identify strains of *S. aureus subsp. aureus* methicillin-resistant.

This medium was provided in Petri plates as a "ready-to-use" medium by S.C. Sanimed International Impex SRL, with the following composition: peptone and yeast extract 30 g/l, sodium chloride 10 g/l, dibasic sodium phosphate 2,5 g/l, selective and matting agents 16,5 g/l, chromogenic with antibiotic mixture 0,8 g/l and agar 15 g/l.
Methicillin-resistant strains were considered strains resistant to at least one of the three antibiotics and strains which had an intermediate behavior to at least two of the three antibiotics and, in the end, 210 strains of coagulase positive and negative staphylococci were selected.

RESULTS AND DISCUSSIONS

The results regarding the behavior to the three beta-lactamases were different and showed that the resistance of the tested strains was maximum to oxacillin and minimum to cefoxitin. The presence of this phenomenon was tested, in parallel, also on the Chromatic™ MRSA medium, the results being compared with the results of the 3 beta-lactams.
On Chromatic™ MRSA, strains of *S. aureus subsp. aureus*, resistant to at least one of the three antibiotics, formed purple to orange colonies, of different nuances and the „non-*S. aureus*"strains of staphylococci, resistant to one of the three antibiotics, formed white or blue colonies.
Based on colony pigmentogenesis, this chromogenic medium allowed the differentiation of *S. aureus subsp. aureus* strains resistant to at least one of the three antibiotics or with intermediate behavior to least two of the three antibiotics. For this species, MRSA strains formed colonies pigmented in purple to purple-orange.
The results showed that all strains included in this species, based on colony pigmentogenesis, were classified as MRSA strains, these results being correlated with the results provided by the Kirby-Bauer disc-diffusion test. Also, based on the results, Chromatic™ MRSA medium can be recommended to identify the *S. aureus subsp. aureus* strains, MRSA type, in the routine diagnosis, which aimes the phenotypic characterization of strains belonging to this species (Table 1).

Table 1. Results obtained on Chromatic MRSA medium

No. crt.	Staphylococcal species	No. of strains	Chromatic MRSA	
			purple/ orange	white/ blue
1.	***S. aureus subsp. aureus***	63	63	-
2.	***S. aureus subsp. anaerobius***	3	1	2
3.	***S. hyicus***	31	-	31
4.	***S. intermedius/ S. pseudintermedius***	21	-	21
5.	***S. caprae***	5	-	5
6.	***S. epidermidis***	15	-	15
7.	***S. equorum***	5	-	5
8.	***S. lentus***	1	-	1
9.	***S. sciuri***	30	-	30
10.	***S. xylosus***	36	-	36
TOTAL		210	64	146

The strains included in 8 staphylococci species, respectively 2 positive coagulase and 6 negative coagulase, formed, on this chromogenic medium, blue or white colonies and, based on the recommendations of the producing company, could be considered strains resistant to methicillin or oxacillin. Six strains of staphylococci didn't grow on this medium, which can act selectively to some methicillin or oxacillin susceptible strains.
The results showed that staphylococci strains, included in the „non-*S. aureus*" group, resistant to methicillin and oxacillin, can be distinguished by MRSA strains with this chromogenic medium. These cultural aspects can be used as phenotypic tests in the routine diagnosis, regarding the rapid differentiation of the two staphylococci groups, namely *S. aureus subsp. aureus* and the group "non-*S. aureus*".
In the case of *S. aureus subsp. anaerobius* species, a strain formed purple-orange colonies, thus classified as MRSA type and two strains formed blue colonies, included in the „non-*S. aureus*" group.
The results obtained from these two phenotypic tests, the Kirby-Bauer disc-diffusion test with methicillin, oxacillin and cefoxitin biodiscs, correlated with chromogenic Chromatic™ MRSA medium, revealed that the phenomenon commonly referred to as methicillin resistance is present both in the strains by *S. aureus subsp. aureus*, as well as strains included in the group "non-*S. aureus*". This group included

three positive coagulase staphylococci species and all negative coagulase staphylococci species, some low pathogenic or non-pathogenic. The methicillin-resistant strains included in these species constitute a reservoir of genetic elements coding this phenomenon (SCC*mec*), which is in permanent extension.
Staphylococci, generically called methicillin-resistant, are considered zoonotic risk bacteria, because this phenomenon is associated with multiple antibiotic resistance. Circulation of methicillin-resistant strains is monitored both in human medicine and veterinary medicine and the human-animal-human circuit is a major concern for public health.
The frequency of the methicillin resistance phenomenon is studied in positive and negative coagulase staphylococci, in *S. aureus subsp. aureus* and „non-*S. aureus*" staphylococci, as well as in *S. aureus* strains referred to as "Livestock Associated-Methicillin Resistant *S. aureus*" and "Community-Associated Methicillin Resistant *S. aureus*" (Feingold B. J. et al., 2012;, Wan M. T. et al., 2012).
For this purpose the Kirby-Bauer phenotypic method, the genotypic method for SCC*mec* detection and chromogenic media are used. The use of chromogenic media is a rapid phenotypic method used in both routine diagnosis and screening research (Dupieux C. et al., 2017).
Similar results to those obtained in the own research have been reported by authors who have used different chromogenic media to detect the methicillin resistance to staphylococci isolated from farm animals for, pets and veterinary clinics (Graveland H. et al., 2009; Pletinckx L. J. et al., 2012).

CONCLUSIONS

Based on colony pigmentogenesis, the Chromatic^{TM} MRSA medium allowed the differentiation of *S. aureus subsp. aureus* strains resistant to at least one of the three antibiotics, since only the strains included in this species produce purple-orange colonies, considered as MRSA colonies.
The results obtained using the two phenotypic tests revealed the phenomenon of methicillin resistance both in *S. aureus subsp. aureus* strains and strains included in the „non-*S. aureus*" group, which confirms the animal-human epidemiological circuit of these strains in both directions.
The Chromatic^{TM} MRSA medium can be used in the routine diagnosis to monitor the frequency of the methicillin-resistant strains

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