

Research on the Phenotypic Characterization of Mrsa Strains Isolated from Animals

IuliaMaria BUCUR*, Viorel HERMAN, Corina PASCU, Ionica IANCU, Janos DEGI and Nicolae CĂTANA

Faculty of Veterinary Medicine, Timișoara
*corresponding author: bucur_iulia@ymail.com

Bulletin UASVM Veterinary Medicine 74(1) / 2017,
Print ISSN 1843-5270; Electronic ISSN 1843-5378
DOI:10.15835/buasvmcn-vm: 12610

Abstract

Currently, at the staphylococci isolated from animals with different diseases, as well as in humans, are monitored the MRSA type strains (Methicillin Resistant *Staphylococcus aureus*), as the methicillin resistance is associated with the resistance to other antibiotic groups. Methicillin resistance is encoded by *mec* staphylococcal chromosomal cassettes (SCC*mec*), which are islands of resistance. The research was made in order to characterize and identify phenotypically the MRSA staphylococci strains isolated from animals.

Researches were made on 240 coagulase positive and coagulase negative strains of staphylococci. Mannitol fermentation was tested on Chapman medium, free coagulase was revealed on Baird-Parker medium and to identify *S. aureus subsp. aureus* was used the chromogenic medium Chromatic Staph. Methicillin-resistant strains were detected by disc diffusion method, using biodiscs with methicillin, oxacillin and ceftiofur. Also, to identify the MRSA strains, was used the chromogenic medium Chromatic MRSA.

The isolates were positive to mannitol and produced complete haemolysis or were unhaemolytic. A number of 44 strains produced free coagulase on Baird-Parker medium, considered coagulase positive strains, while 196 were coagulase negative strains. The isolates conducted differently to methicillin: 22,08% of strains were resistant, 51,25% of strains were susceptible and 26,66% had intermediate resistance, while 42,91% of strains were resistant to oxacillin. The increased frequency of methicillin-resistant strains of staphylococci and, particularly, MRSA strains, determined using the ceftiofur disk diffusion test, which is more reliable than methicillin and oxacillin. On the MRSA chromogenic medium, the methicillin-resistant strains of staphylococci formed colonies with pigment from mauve to orange mauve.

The results obtained by using the disk diffusion test on resistance patterns to 3 beta-lactams, resistant to penicillinase, indicated a different frequency of the resistant strains to these antibiotics. Ceftiofur disk diffusion test revealed a frequency of 2,51% resistant strains, considered to be MRSA strains.

Keywords: *chromogen, methicillin, MRSA, resistance, staphylococcus*

INTRODUCTION

Staphylococci strains are considered strains with a highly zoonotic risk that have a complex epidemiologic circuit, because it can present resistance phenotypes to methicillin

Currently, at the staphylococci isolated from animals with different diseases, as well as in humans, are monitored the MRSA type strains (Methicillin Resistant *Staphylococcus*

aureus), because the methicillin resistance is associated with resistance to other antibiotic groups (Bicheru *et al.*, 2014; Codiță *et al.*, 2008).

Resistance to methicillin is encoded by *mec* staphylococcal chromosomal cassettes (SCC*mec*), which are islands of resistance, composed of: *mecA*, *mecR1* and *mecO* genes, a complex coding the *crrA* and *crrB* recombinases, Tn544 transposon and a copy of the plasmid pUB110. Chromosomal

Tab. 1. Results regarding the antibioresistance testing

Specification	METHICILLIN		OXACILLIN		CEFOXITIN	
	No	%	No	%	No	%
CPS susceptible strains	29	65,91%	15	34,09%	43	97,73%
CNS susceptible strains	94	47,96%	43	21,94%	180	91,84%
TOTAL	123	51,25%	58	24,16%	223	92,92%
CPS intermediate strains	6	13,64%	11	25%	1	2,27%
CNS intermediate strains	58	29,59%	68	28,33%	10	5,10%
TOTAL	64	26,67%	79	32,92%	11	4,58%
<i>CPS resistant strains</i>	9	20,45%	18	40,91%	0	0%
<i>CNS resistant strains</i>	44	22,45%	85	35,42%	6	3,06%
TOTAL	53	22,08%	103	42,92%	6	2,5%

cassettes can be transmitted, and as a result MRSA type strains are becoming more frequently. These strains can be identified by molecular biology tests and tests that reveal several phenotypic characteristics (Bicheru *et al.*, 2014; Codiță *et al.*, 2008; Pletinckx *et al.*, 2013).

The research was made in order to characterize and identify phenotypically the MRSA staphylococci strains isolated from animals.

MATERIALS AND METHODS

The research was made on a total of 240 coagulase positive and coagulase negative strains of staphylococci, isolated from animals. Samples with pathological material were taken with sterile swabs. In order to obtain the primary cultures the sowings were made in peptone water and incubated at 37°C, for 18-20 hours, under aerobic conditions. Chapman medium was used to obtain pure cultures of staphylococci, which excluded non halotolerant bacteria and highlighted mannitol fermentation.

Free coagulase was revealed on Baird-Parker medium and, for the identification of *S. aureus subsp. aureus*, was used the chromogenic medium Chromatic™ Staph aureus (Codiță, 2008).

Methicillin-resistant strains were detected by disc diffusion method, using biodiscs with methicillin (5 µg), oxacillin (1 µg) and cefoxitin (30 µg). Also, a chromogenic medium (Chromatic™ MRSA) was used to identify the MRSA strains.

RESULTS AND DISCUSSION

The results regarding the resistance to these three β-lactams were different, but however,

confirmed that in coagulase positive and negative staphylococci, isolated from animals, the resistance phenotypes to these antibiotics are present.

A number of 44 coagulase positive strains and 196 coagulase negative strains were identified using Baird-Parker medium, strains tested for resistance to these three β-lactams.

On Chromatic™ Staph aureus medium used for a rapid discrimination of *S. aureus subsp. aureus* strains, the isolates formed pink-purplish colonies. Based on this phenotypic character, 47 strains (19,58%) were included in this species.

In Table 1 are presented the results from testing the behavior of coagulase positive and negative staphylococci towards these three β-lactams.

Coagulase positive staphylococci strains behavior to methicillin, oxacillin and cefoxitin was variable, as follows: 20,45% of strains were resistant to methicillin, 40,91% were resistant to oxacillin, while no strain was resistant to cefoxitin. Also, **coagulase negative staphylococci** strains behavior to methicillin, oxacillin and cefoxitin was different: 22,45% of strains were resistant to methicillin, 35,42% were resistant to oxacillin, while only 3,06 % of strains were resistant to cefoxitin.

Coagulase positive and coagulase negative staphylococci strains, had a different behavior to **methicillin**, an antibiotic frequently used for detection of methicillin-resistance phenomenon. Thus, 22,08% of the tested strains were resistant to this antibiotic, 51,25% of the tested strains were susceptible and 29,59% had an intermediate resistance.

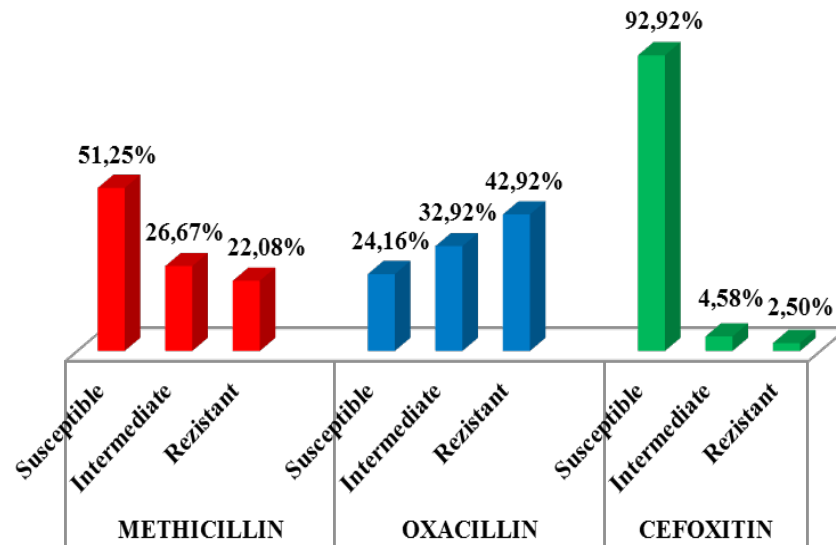


Fig. 1. Resistance phenotypes frequency of CPS and CNS strains

In order to detect the cross-resistance of staphylococci to penicillins resistant to penicillinase, **oxacillin** was recommended, both for its stability and for reproductibility of results. Thereby, the results were as follows: 42,92% were resistant strains, 21,94% were susceptible strains and 32,92% had an intermediate behavior.

The increased frequency of methicillin resistant staphylococci strains, in particular, of *S. aureus subsp. aureus* strains, namely MRSA strains, determined the use of disk diffusion test to **cefoxitin**, an antibiotic more reliable than methicillin and oxacillin. The results obtained on testing the isolates to cefoxitin were: 2,5% resistant strains, 92,92% susceptible strains and 4,58% of strains had an intermediate behavior (Fig. 1.).

On Chromatic™ MRSA medium staphylococci in this group formed colonies of mauve to orange-mauve color. With this culture medium, 39 strains (16,25%) were classified as MRSA strains.

Methicillin resistant staphylococci are considered bacteria with zoonotic risk, and the study of animal-human-animal circuit to these bacteria are a major concern for public health. Strains of methicillin resistant staphylococci are frequently isolated, both from farm animals and from pets, but as researches were conducted, the phenomenon of methicillin resistance is particularly aimed at two large groups of staphylococci, respectively coagulase positive and coagulase negative (Boost *et al.*, 2012;

Gordoncillo *et al.*, 2012; Vanderhaeghen *et al.*, 2012; Vanderhaeghen *et al.*, 2013).

Lately, methicillin resistant staphylococci are frequently isolated from swine in intensive growth. These strains have a growing frequency and found in pigs in many countries.

Based on research conducted by numerous collectives, MRSA-ST398 strain was identified, which produce frequent infections in humans (caretakers, farmers, processors and veterinary personnel) and recognized as a highly professional risk strain (Lim *et al.*, 2012).

The importance of MRSA strains to public health and the increasing frequency of these strains require rapid detection methods, both phenotypic and genotypic, like the use of chromogenic media and some variants of the PCR test (Bicheru *et al.*, 2014; Gunderson *et al.*, 2016; Vanderhaeghen *et al.*, 2012; Vanderhaeghen *et al.*, 2013).

Own research confirms the data in the literature regarding the frequency of resistance phenotypes to those three β -lactams and, in particular, the frequency and zoonotic risk of MRSA strains.

CONCLUSIONS

A number of 240 strains of staphylococci were isolated and standardized based on the cultural, morphological, tinctorial, hemolytic and mannitol fermentation characters from the samples of pathological material.

Using the chromogenic media, were discriminated *S. aureus subsp. aureus* strains and the strains belonging to the MRSA group.

The results, obtained by disk diffusion method on the resistance patterns to three beta-lactams resistant to penicillinase, indicated a different frequency of the resistant strains to these three antibiotics.

The disk diffusion test with oxacillin detected a higher frequency of the resistant strains compared with the methicillin disk diffusion test.

The test using cefoxitin disk diffusion revealed a frequency of 2,51 % resistant strains, that are considered MRSA strains.

Acknowledgement: This research work was carried out with the support of the project „Dezvoltareainfrastructurii de cercetare, educație și servicii în domeniile medicinei veterinare și tehnologiilor inovative pentru RO 05, cod SMIS-CSNR 2669”.

REFERENCES

1. Bicheru S, Necșulescu M, Cumpănășoiu B, Tîrziu E, Popescu D, Ionescu L, Dumitrescu G, Necșulescu A (2014). PCR for the identification of methicillin resistant staphylococcus aureus (MRSA) strains using primers specific for Scmec elements, *Lucr. Șt. Med. Vet.* vol XLVII(2) Timișoara.
2. Boost MV, Ho J, Guardabassi L, O'donoghue MM (2012). High methicillin-resistant Staphylococcus aureus carriage in intensive pig farms in southern China, *Veterinary Record* 171(6): 156.
3. Codiță I (2008). Identificarea stafilococilor, *Tratat de microbiologie clinic sub redacția Buiuc D, Neaguț M. Ed. Medicală București* 592-606.
4. Codiță I, Buiuc D (2008). Determinarea sensibilității la antibiotice: teste calitative , *Tratat de microbiologie clinică, ediția a II-a, sub redacția Buiuc D, Neaguț M, Ed. Medicală București* 453-482.
5. Gorduncillo MJ, Abdujamilova N, Perri M, Donabedian S, Zervos M, Bartlett P (2012). Detection of methicillin-resistant Staphylococcus aureus (MRSA) in backyard pigs and their owners, Michigan, USA, *Zoonoses Public Health* 59(3):212-216.
6. Gunderson CG, Holleck JL, Chang JJ, Merchant N, Lin S, Gupta S (2016). Diagnostic accuracy of methicillin-resistant Staphylococcus aureus nasal colonization to predict methicillin-resistant S. aureus soft tissue infections, *American Journal of Infection Control*(16):302-353.
7. Lim SK, Nam HM, Jang GC, Lee HS, Jung SC, Kwak HS (2012). The first detection of methicillin-resistant Staphylococcus aureus ST398 in pigs in Korea, *Veterinary Microbiology* 155(1):88-92.
8. Pletinckx LJ, Verheghe M, Crombé F, Dewulf J, DeBleecker Y, Rasschaert G, Butaye P, Goddeeris BM, De Man I (2013). Evidence of possible methicillin-resistant Staphylococcus aureus ST398 spread between pigs and other animals and people residing on the same farm, *Preventive Veterinary Medicine* 109(3-4):293-303.
9. Vanderhaeghen W, Van De Velde E, Crombé F, Polis I, Hermans K, Haesebrouck F, Butaye P (2012). Screening for methicillin-resistant staphylococci in dogs admitted to a veterinary teaching hospital, *Research in Veterinary Science* 93(1):133-136.
10. Vanderhaeghen W, Vandendriessche S, Crombé F, Nemeghaire S, Dispas M, Denis O, Hermans K, Haesebrouck F, Butaye P (2013). Characterization of methicillin-resistant non-Staphylococcus aureus staphylococci carriage isolates from different bovine populations, *The Journal of Antimicrobial Chemotherapy* 68(2):300-307.