VERSITA

Acta Veterinaria-Beograd 2014, 64 (1), 115-123
UDK: 637.12.065:579.86 ; 636.2.09:618.19-002
DOI: 10.2478/acve-2014-0012

Research article

CHARACTERISTICS OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM MILK IN CASES OF SUBCLINICAL MASTITIS

RAJIĆ SAVIĆ Nataša[1*], KATIĆ Vera[2], VELEBIT Branko[3]

[1]Ekolab Ltd. Padinska Skela, Serbia; [2]University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia; [3]Institute of Meat Hygiene and Technology, Belgrade, Serbia

(Received 03 January 2014; Accepted 07 February 2014)

Coagulase-positive staphylococci are the most common pathogen causing subclinical mastitis in cows. Their main characteristic is a high virulence which leads to chronic infection. A total of 213 isolates of coagulase-positive staphylococci were tested. The majority of isolates (58%) formed a gold pigment, then light gold (28%), white gold (8%), golden gray, creamy white and white (2%). The majority of isolated coagulase-positive staphylococci produce beta hemolysis on esculin blood agar (50%), alpha and beta hemolysis (36%), beta and delta hemolysis (8%), delta hemolysis (4%), and alpha hemolysis (2%). Biochemical and molecular identification was performed by APISTAPH and multiplex PCR method. The majority of isolates were identified as *S. aureus* (88%), *S. chromogenes* (4%), and 2% of the isolates were identified as *S. lentus, S. sciuri, S. xylosus, S. intermedius* by APISTAPH. Antimicrobial susceptibility to penicillin G, was found by the Kirby Bauer disk diffusion method to be resistant (62.44%). For disc diffusion penicillin G sensitive isolates the minimal inhibitory concentration (MIC) was established for MIC_{50} and MIC_{90} as 0.003 mg/ml and 2μg/ml, respectively. For disc diffusion penicillin G resistant isolates MIC_{50} and MIC_{90} was 1.0μg/ml and 16μg/ml, respectively. The study of phenotypic resistance to methicillin, as recommended by CLSI, established resistance to oxacillin in 5.26% of the isolates, while no resistance was found to cefoxitine. None of the tested isolates have the *mec*A gene.

Key words: coagulase positive staphylococci, hemolysis, identification, methicillin, penicillin, pigment

INTRODUCTION

Numerous studies showed that coagulase-positive staphylococci are the main subclinical mastitis pathogens [1,2]. The main reservoirs of coagulase- positive staphylococci are animals that have a latent udder infection, and they can be transmitted from one animal to another during milking. Declining resistance of animals can result in the inflammation of the udder, usually manifested in a subclinical form.

Primary identification of pathogenic staphylococci is based upon their ability to produce colored colonies, red blood cells haemolysis and coagulate rabbit plasma.

*Corresponding author: e-mail: nrajicsavic@yahoo.com

Most pathogenic staphylococci produce a number of carotenoid compounds that have a yellow color as the end product of the synthesis of the deep golden pigment staphyloxantine [3]. Staphylococci that produce the gold pigment are better protected in situations of oxidative stress during phagocytosis, but are significantly more resistant to antimicrobials [3,4].

Haemolysins are important virulence factor. Phenotypic hemolysis indicates that the strain is active in the synthesis of enzymes that damage the tissue and allows them to survive and multiply. Alpha hemolysin shows activity against a variety of cell types and plays a major role in the development of pathological processes in tissues [5,6]. Beta hemolysin is a sphingomyelinase C enzyme and plays an active role in the pathogenesis of mastitis caused by *S. aureus.* Gamma hemolysin acts on neutrophils and macrophages and delta hemolysin acts as a surface-active substance. They are frequently found strains that produce multiple types of hemolysin, mostly alpha and beta, as well as various combinations of hemolysins: alpha+beta+delta and beta+delta.

The most dominant coagulase positive staphylococci is *S. aureus*, but they are other staphylococci that can coagulate rabbit plasma: *S. hyicus, S. intermeduius, S. lutrae, S. pseudointermeduis.* [5,7,8] One of the reasons for treatment failure is the emergence of strains that produce beta lactamase and inactive penicillinase sensitive penicillins. Numerous reports indicate the increasing resistance of coagulase- positive staphylococci to antibiotics, especially penicillin [6,9,10]. A high percentage of *S. aureus* isolates resistant to penicillin was detected in all European countries except France, where the percentage of isolates resistant to penicillin was less than 10% [11]. Resistant strains can exchange genes with resistance isolates in humans [12].

In the recent years, there are published reports on findings of staphylococci isolated from the milk of cows suffering from mastitis that are resistant to methicillin (MRSA). Those staphylococci have the *mec*A gene, which codes for altered penicillin- binding protein 2 (PBP2). These isolates are often resistant to many antimicrobials used for the treatment of mastitis.

Methicillin resistance in strains of *S. aureus* isolated in mastitis cases, has been found in a small number of samples [2,13,14]. Due to the contact between humans and cows, there is a risk of transmission of methicillin-resistant staphylococci in humans.

The aim of this study was to examine the basic characteristics of coagulase- positive staphylococci as a cause of subclinical mastitis and the risk they pose to the environment and people.

MATERIALS AND METHODS

A total of 213 coagulase positive staphylococci isolated from subclinical mastits milk samples were collected. The isolated staphylococci were Gram positive, catalase positive, have the ability to pigment and produce haemolysins, as well as coagulate rabbit plasma. Biochemical identification of the strains was made with APIStaph bioMérieux identification systems, and molecular identification and *mec*A gene detection was carried out by PCR.

All isolates were tested for penicillin sensitivity using 6μg (Torlak) discs by Kirby Bauer

disk diffusion method on Mueller-Hinton agar Becton Dicinson and the results were recorded after 24h incubation at 37°C. The zone diameter was determinined and interpreted according to producer's recommendation.

Macrodilution method for the determinination of the minimal inhibitory concentration (MIC) of penicillin was performed by the cation aduced Mueller-Hinton broth Becton Dicinson [15].

The isolates which were evaluated by disk diffusion method as sensitive to penicillin G were inoculated in the broth with an initial concentration range of 8μg/ml penicillin G, and dilutions of 4, 2, 1, 0.5, 0.25, 0.125, 0.06 and 0.03 μg /ml were made, while the isolates resistant to penicillin G were formed from an initial concentration of 128μg/ml penicillin G, and thereon dilutions of 64, 32, 16, 8, 4, 2, and 1μg/ml were made. Broths with penicillin G were inoculated with bacterial suspension and incubated during 24h at 37°C.

Phenotype testing for the presence of the *mec*A gene was performed by disc diffusion method on Mueller-Hinton plates Becton Dicinson using 1μg oxacilin (Becton-Dikinson) and 30μg cefoxitine (Becton-Dikinson) discs recomanded by CLSI [15].

Molecular identification of selected coagulase-positive staphylococci and detection of the *mec*A gene was performed by multiplex PCR method. To determine the 16S rRNA characteristic for the genus *Staphylococcus* were used 16S- F 5'-GTGCCAGCAGCCGCGGTAA-3' and 16S-R 5'-AGACCCGGGAACGTATTCAC-3' for detection of *nuc* genes characteristic of *Staphylococcus aureus* were used nuc -f 5' - TCAGCAAATGCATCACAAACAG -3 ' and 5' -r *nuc* - CGTAAATGCACTTGCTTCAGG -3, while the detection of the *mec*A gene was done with the *mec*A -F 5'-GGGATCATAGCGTCATTATTC-3' and 5'-r *mec*A-AACGATTGTGACACGATAGCC-3'. ATCC 43300 *Staphylococcus aureus MRSA Positive* was used as the control strain.

After preparation, amplification was carried out as follows: an initial denaturation step at 95°C for 5 min; 10 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 60sec; and a final elongation at 72°C for 7 min. After amplification was performed gel electrophoresis was performed, and the gel bands were visualized.

RESULTS

Primary identification of the 213 coagulase-positive staphylococci isolates was performed on blood agar colony based on color and hemolysis and tube tests. Based on pigment and hemolysis features, 50 typical strains were allocated. Results of phenotypic characterization are shown in Table 1.

Identification of selected staphylococci isolates was made on the basis of the biochemical properties using APISTAPH and multiplex PCR method. The results of biochemical and molecular identification are shown in Table 2.

Table 1. Colors and hemolytic colonies typical for strains of coagulase- positive staphylococci

Colony pigment	No. isolates	%	Hemolysis	No. isolates	%
Golden gray	1	2	α	1	2
Gold	29	58	β	25	50
Light gold	14	28	Δ	2	4
White gold	4	8	α + β	18	36
Creamy white	1	2	Δ + β	4	8
Whitish	1	2	Total	50	100
Total	50	100			

Table 2. Identification of selected isolates of coagulase- positive staphylococci by biochemical and molecular methods

Species of staphylococci based on the API tests	No of isolates	16S rRNK		nuc gene	
		Number	%	Number	%
S. aureus	44	44	100	39	88.64
S. chromogenes	2	0	2	0	100
S. intermedius	1	0	1	0	100
S. lentus	1	0	1	0	100
S. sciuri	1	0	0	0	0
S. xylosus	1	0	1	0	100

All selected isolates of staphylococci were tested for penicillin G susceptibillity by disc diffusion and macrodilution method (Table 3).

Table 3. Antimicrobial testing to penicillin G by disc diffusion and macrodilution method

Disc diffusion method	No. isolates	%	Macrodillution method	MIC_{50}	MIC_{90}
Sensitive	56	26.29	Penicillin sensitive	0.003μg/ml	2μg/ml
Intermediate sensitive	24	11.26			
Resistant	133	62.44	Penicillin resistant	1μg/ml	16μg/ml

Fifty selected isolates, also, were tested for the presence of the *mec*A gene determining resistance to oxacillin and cefoxitin method recommended by the CLSI and by multiplex PCR method (Table 4).

Table 4. Investigation on phenotypic and genotypic resistance to methicillin in isolates of coagulase- positive staphylococci that were resistant to penicillin G

No of tested isolates	Oxacillin resistant		Cefoxitine resistant		mecA gene		
	No	%	No	%	No of tested isolates	No positive	%
133	7	5.26	0	0	50	0	0

DISCUSSION

Bacteria able to produce pigments are more resistant to host defense factors. Most of our isolates (58%) had a golden pigment, then light gold (28%), white gold (8%) and one isolate (2%) had a golden gray, creamy, white and whitish pigment. Similar results were obtained by Begum et al. [4] who found that coagulase positive staphylococci isolated from subclinical mastitis milk samples produced a golden-yellow (25.71%), yellow (51.43%) and white pigment (22.86%). Takeshige et al. [16] reported that 86.2% of the *S. aureus* isolated from mastitis milk samples produced the yellow pigment.

The majority of coagulase-positive staphylococci, isolated in this investigation, produce beta hemolysis on blood agar (50%), alpha and beta hemolysis (36%), beta and delta hemolysis (8%), delta hemolysis (4%), and alpha hemolysis (2%). Franco et al. [6] found that *S. aureus* produces beta hemolysis in 29 % of cases, and alpha and beta hemolysis in 37% of cases. Silva et al. [17] found that 43.3% of isolates of coagulase- positive staphylococci on blood agar gives beta hemolysis, and 26.8% of isolates give alpha and beta hemolysis.

Biochemical identification of the selected 50 isolates of coagulase-positive staphylococci by APIStaph systems revealed that 44 (88%) belong to *S. aureus*, and 6 (12%) belong to other species of coagulase-positive staphylococci. Cappuro et al. [8] reported that 97% of coagulase-positive staphylococci isolates were biochemically identified as *S. aureus* and Da Costa et al. [18] reported in 98.25% of isolates coagulase-positive staphylococci and Robertson et al. [7] identified as *S. aureus* in 82.1% of coagulase-positive staphylococci isolates.

Other species of staphylococci isolated on the basis of biochemical characteristics in two isolates (4%) were identified as *S. chromogenes*, and one isolate as *S intermedius* (2%), *S. xylosus* (2%), *S. sciuri* (2%) and *S. lentus* (2%). According to the results of Langlois et al. [19] APIStaph identification systems in 99.2% of cases is reliable for the identification of *S. aureus,* but only 41.8% for other coagulase-positive staphylococci. Biochemical identication systems are created for the identification human *S. aureus,* and, nonaureus coagulase positive staphylococci can be misinterpreted as coagulase negative staphylococci.

Of the 44 isolatestested with APIStaph 43 were identified as *S. aureus*, which was confirmed by PCR. The results of the present study are in agreement with the results of other authors [18]. In the case of other types of staphylococci identification results is less precise.

The most common mechanism of resistance to antibiotics of coagulase-positive staphylococci is the synthesis of beta lactamases that inactivate penicillinase sensitive antibiotics. Resistance to beta-lactam antibiotics in coagulase- positive staphylococci is increasing and following resistance in human isolates. Some studies show that penicillin disk diffusion method showes deviation in the case of sensitive beta-lactam antibiotics in relation to the dilution method.

A similar proportion of isolates of *S. aureus* resistant to penicillin was found by Güler et al. [20] with 63.3% of the isolates, Kalmus et al. [21] with 61.4% of the isolates and Malinowski et al. [10] with 66.5% of the isolates. Turutoglu et al. [22] determined

resistance to penicillin in 69% of isolates, with most resistant isolates (82.2%) producing beta lactamase. Some authors reported *S. aureus* resistance to penicillin determined by disk diffusion method to range from 87,5% to 95% [5,23,24].

When isolates were sensitive to penicillin, dilution method found MIC_{50} 0,003 μg/ml penicillin, indicating, as interpreted by CLSI, that these isolates were sensitive to penicillin that do not synthesize beta lactamase, and the MIC_{90} was 2μg/ml, indicating that these isolates resistant to penicillin, and there is no agreement between the disk diffusion and dilution method. Disagreement between the disk diffusion and dilution methods were found Gianneechini et al. [2] that the disk diffusion method demonstrated 46,1% of the isolates of *S. aureus* resistant to penicillin and following minimum inhibitory concentrations determined MIC_{50} 0,5μg/ml and MIC_{90}>8μg/ml. Similar results were obtained by Watts et al. [25] showed in the strains of *S. aureus* isolated in mastitis cases, which produce beta lactamase a $MIC_{50} \leq$ 0,06μg/ml and MIC_{90} 0,25μg/ml, and strains producing beta lactamase MIC_{50} 0,5μg/ml and MIC_{90} 16μg/ml.

MRSA isolated in cases of mastitis was confirmed in only two EU countries, Spain (3.7%) and in France, (8.3%) isolates. In this study 5.26% isolates were resistant to oxacillin. None of the tested isolates were resistant to cefoxitin, nor did the PCR method prove the presence of the *mec*A gene. Results similar to ours were reported by Kaya et al. [26] who found one isolate susceptible to oxacillin, but have not shown the presence of genes for methicillin resistance. There are some reports on the presence of the *mec*A gene in coagulase positive staphylococci isolated from milk samples of cows suffering from mastitis. Saini et al. [27] found the *mec*A gene in one *S. aureus* isolate, which was sensitive to oxacillin and Moon et al. [28] detected *mec*A gene in 1.54% isolates *S. aureus* resistant to oxacillin.

CONCLUSION

The majority of the coagulase positive staphylococci isolated from milk in subclinical mastits cases had the ability to produce gold pigment and hemolysins, of which the most frequent was beta hemolysin. The most common isolates were *Staphylococcus aureus* identified by biochemical and molecular methods. A high percentage of isolates were resistant to penicillin. None of the isolates were resistant to methicillin.

ACKNOWLEDGMENTS

The investigation was supported by the Ministry of Science and Technology Development, Republic of Serbia. Project No TR 31066.

REFERENCES

1. Malinowski E, Lassa H, Kllossowska A, Smulski S, Markiewicz H, Kaczmarowski M: Etiological agents of dairy cows' mastitis in western part of Poland**.** Pol J Vet Sci 2006, 9(3):191-4.
2. Gianneechini RE, Concha C and Franklin A: Antimicrobial susceptibility of udder

pathogens isolated from dairy herds in the west littoral region of Uruguay. Acta Vet Scand 2002, 43:31-41.

3. Wileland B, Feil C, Gloria-Maercker E, Thumm G, Lechner M, Bravo JM, Poralla K, Gotz F: Genetic and biochemical analyses of the biosynthesis of the yellow carotenoid 4,4'-diaponeurosporene of *Staphylococcus aureus*. J Bacteriol 1994, 176(24):7719-7726.
4. Begum HA, Uddin MS, Islam MJ, Nazir KHMNH, Islam MA, Rahman MT: Detection of biofilm producing coagulase positive *Staphylococcus aureus* from bovine mastitis, their pigment production, hemolytic activity and antibiotic sensitivity pattern. J Bangladesh Soc Agric Sc. Technol 2007, 4(1-2): 97-100.
5. Ebrahimi A, Akhavan TM: Characteristics of staphylococci isolated from clinical and subclinical mastitis cows in Shahrekord, Iran. Iran J Vet Res 2009, 10(28): 273-277.
6. Franco G, Julio C, González V Libertad, Gómez M, Sandra C, Carrillo G, Juan M, Ramírez C, José J: Virulence factors analysis of *Staphylococcus aureus* isolated from bovine mastitis in México. e-Gnosis 2008, 6:1-9.
7. Roberson JR, Fox LK, Hancock DD, Gay JM, Besser TE: Prevalence of coagulase-positive staphylococci, other than *Staphylococcus aureus*, in bovine mastitis. Am J Vet Res 1996, 57(1):54-8.
8. Capurro A, Concha C, Nilsson L, Ostensson K: Identification of coagulase-positive staphylococci isolated from bovine milk. Acta Vet Scand 1999, 40:315-21.
9. National mastitis council research committee report bovine mastitis pathogens and trends in resistance to antibacterial drugs. NMC Annual Meeting Proceedings. 2004, 400-414.
10. Malinowski E, Klossowska A, Kaczmarowski M, Lassa H, Kuzma K: Antimicrobial susceptibility of staphylococci isolated from affected with mastitis cow. Bull Vet Inst Pulawy 2002, 46:289-94.
11. Hendriksen RS, Mevius DJ, Schroeter A, Teale C, Meunier D, Butaye P, Franco A, Utinane A, Amado A, Moreno M, Greko C, Strk K, Berghold C, Myllyniemi AL, Wasyl D, Sunde M, Aarestrup FM: Prevalence of antimicrobial resistance among bacterial pathogens isolated from cattle in different European countries: 2002-2004. Acta Vet Scand 2008, 8(50):28.
12. Moon JS, Lee AR, Jaw SH, Kang HM, Joo YS, Park YH, Kim MN, Koo HC: Comparison of antibiogram, staphylococcal enterotoxin productivity, and coagulase genotypes among *Staphylococcus aureus* isolated from animal and vegetable sources in Korea. J Food Prot 2007, 70:2541-2548.
13. Turutoglu H, Ercelik S, Ozturk D: Antibiotic resistance of *Staphylococcus aureus* and coagulase-negative staphilococci isolated from bovine mastitis. Bull Vet Inst Pulawy 2006, 50:41-45.
14. Europen Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. European Food Safety Authority 2, 3 European Centre for Disease Prevention and Control, EFSA Journal 2012, 10(3): 2598.
15. CLSI (Clinical and Laboratory Standard Institute): Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. 2006, January 26(3).
16. Takeshige K, Watanabe K, Igarashi H, Shingaki M, Terayama T: Detection of *Staphylococcus aureus* in bovine mastitis and some characteristics with special reference to enterotoxin producibility and coagulase types of isolates. Nihon JuigakuZasshi 1983, 45(3):355-362.
17. Silva Padilha W, Destro MT: Biochemical characteristic of typical and atypical *Staphylococcus aureus* in mastitic milk and environmental samples of Brazilian dairy farms. Braz. J. Microbiol

2000, 31(2):103-106.
18. Da Costa GM, Vilela Paiva L, Hilsdorf Piccoli R, Figueiredo Demétrio J, Ulisses de Pádua P, da SilvaN: Evaluation of a simplified key for the identification of coagulase-positive Staphylococcus isolated from bovine mastitis. Acta Biol Sci 2010, 32(4):403-406.
19. Langlos EB, Robert J. Harmon and Akers K: Identification of Staphylococcus Species of Bovine Origin with the API Staph-Ident System. J Clin Microbiol 1983, 18:1212-1219.
20. Güler L, Ok Ü, Gündüz K, Gülcü Y, Hadimli HH: Antimicrobial susceptibility and coagulase gene typing of *Staphylococcus aureus* isolated from bovine clinical mastitis cases in Turkey. J Dairy Sci 2005, 88(9):3149-3154.
21. Kalmus P**,** Birgit A**,** Age K**,** Toomas O, Kalle K: Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. Acta Vet Scand 2011, 53:4.
22. Turutoglu H, Mudul S, Pehlivanoglu F: Antibiotic susceptibility and β-actamase prevalence for staphylococci isolated from bovine mastitic milk samples. Acta Vet (Beograd) 2002, 52:337-344.
23. Bengtsson B , Helle EU, Torkel E, Karin A, Nilsson-ÖstM, Karin PW: Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. Vet Microbiol 2009, 136:142-149.
24. Kirkan S, Goksoy EO, Kaya O: Identification and antimicrobial susceptibility of *Staphylococcus aureus* and coagulase negative staphylococci from bovine mastitis in the aydýn region of Turkey. J Vet Anim Sci 2005, 29:791-96.
25. Watts J, Yancey RJ: Comparison of success of antibiotic therapy during lactation and results of antimicrobial susceptibility tests for bovine mastitis. J Dairy Sci 1997, 80(2):313-317.
26. Kaya EG, Esra K, Serap Y, Mihriban Y: Evaluation of phenotypic and genotypic methods for detection of methicillin resistance in Staphylococcus. African J Microbiol Res 2009, 3(12): 925-29.
27. Saini V, McClure JT, Scholl DT, DeVries TJ, Barkema HW: Herd-level association between antimicrobial use and antimicrobial resistance in bovine mastitis staphylococcus aureus isolates on Canadian dairy farms. J Dairy Sci 2012, 95:1921–1929
28. Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH, Park YH, Joo YS, Koo HC: Phenotypic and Genetic Antibiogram of Methicillin-Resistant Staphylococci Isolated from Bovine Mastitis in Korea. J. Dairy Sci 2009, 90:1176-85.

KARAKTERISTIKE KOAGULAZA POZITIVNIH STAFILOKOKA IZOLOVANIH IZ MLEKA KRAVA U SLUČAJEVIMA SUBKLINIČKOG MASTITISA

RAJIĆ SAVIĆ Nataša, KATIĆ Vera, VELEBIT Branko

Koagulaza pozitivne stafilokoke su najčešći uzročnici subkliničkih mastitisa krava. Njihove karakteristike su, ujedno, i faktori virulencije kojima opstaju u vimenu i dovode do hronične infekcije. Ukupno je ispitano 213 izolata koagulaza pozitivnih stafilokoka izolovanih iz mleka krava u slučajevima subkliničkih mastitisa. Najveći

broj izolata koagulaza pozitivnih stafilokoka (58%) je stvarao zlatan pigment, zatim svetlo zlatan(28%), belo zlatan (8%); zlatnosiv, krem beo i beo po 2% ispitanih izolata. Najčešći tip hemolize je beta koji je dokazan kod 50% izolata, alfa i beta kod 36%, beta i delta kod 8%, delta kod 4% i alfa kod 2% ispitanih izolata. Biohemijska i molekularna identifikacija su rađene APISTAPH sistemima i multipleks PCR metodom.Utvrđeno je da se, APISTAPH identifikacionim kitovima, najčešće kao uzročnik javljao *S. aureus* (88% izolata), zatim *S. chromogenes* (4%), a po 2% izolata je identifikovano kao *S. lentus*, *S. sciuri*, *S. xylosus, S. intermedius*. Ispitivanjem osetljivosti na penicilin disk difuzionom Kirby Bauer metodom ustanovljeno je da je 62,44% izolata bilo rezistentno, dok je ispitivanjem minimalne inhibitorne koncetracije (MIC) ustanovljen MIC_{50} i MIC_{90} 0,003μg/ml i 2μg/ml penicilina G kod osetljivih, odnosno 1μg/ml i 16μg/ml penicilina G kod rezistentnih izolata. Ispitivanjem fenotipske rezistencije na meticilin metodom preporučenom od CLSI, ustanovljena je otpornost na oksacilin kod 5,26% izolata, dok na cefoksitin nije ustanovljen nijedan rezistentan izolat. Nijedan ispitivani soj nije imao *mec*A gen.