

SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM BOVINE INTRAMAMMARY INFECTIONS TO DIFFERENT ANTIMICROBIAL AGENTS

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Summary

We have shown the results of routine microbiological testing and susceptibility of *Staphylococcus aureus* isolates that were isolated from cows with inflammation of mammary gland during a five year period. Totally, 2719 strains identified as *Staphylococcus aureus* were routinely tested to twelve antimicrobial agents by the agar-disc diffusion technique. The strains were isolated from 45000 samples of udder secretion on Columbia agar plates containing 5% of defibrinated ovine blood (0.01 ml on a quarter of culture medium). They were identified on the basis of colony morphology, catalase and coagulase tests, a tube coagulase test and the strains from 2002 were identified by the API ID 32 Staph system. Of strains isolated in 2002, 70.77% were penicillin-susceptible, higher than any other year, after that 39.13% of strains in 2007, 37.54% in 2008, 37.96% in 2009, and 44.65% in 2010. Totally, 66.66% of oxacillin-susceptible strains were found in 2007, 67.45% in 2008, 71.52% in 2009 and 88.56% in 2010. For potentiated penicillins like amoxicillin and clavulanic acid 93.85% to 95.65% of susceptible strains were found. For first-generation cephalosporins 36.66%-85.98% of strains demonstrated susceptibility, while for third-generation cephalosporins 100% of strains were susceptible in 2002 and 2007, but 91.07% of isolated strains were susceptible in 2009 and 95.94% in 2010. The strains have shown various susceptibility to tetracyclines, lincosamides, aminoglycosides, fluoroquinolone and sulfonamides. The results show the resistance of strains to all groups of antibiotics.

Keywords: *Staphylococcus aureus*; cow; mastitis; antimicrobial agents

INTRODUCTION

The strain *Staphylococcus aureus* which is reported as the most frequently isolated pathogen in bovine intramammary infection, generally responds poorly to treatment, and can be found in almost all dairy herds. Epidemiological studies revealed the transmission of *Staphylococcus aureus* from cow to cow, the primary source of which is the milk from infected glands, and also from dairy cows to humans and humans to cows [5,6]. For 70 years penicillin has been extensively used for the treatment of intramammary infections and, today a wide range of antimicrobial agents are used during lactation or for dry cow therapy. *Staphylococcus aureus* strains are well adapted for survival and growth in the mammary gland, and with the production of a number of virulence factors (enzymes hyaluronidase, staphylokinase, proteinase), formation of microabscesses, capsules and L-forms and by having the ability to resist phagocytosis and antibiotics, make therapy difficult. Treatment effectiveness depends on many factors such as the choice of antimicrobial agent, tissue damage, duration and severity of infection, changes in the mammary gland and general condition of the cow. The research have shown that dry cow treatment is more effective than during lactation period. The successful treatment of chronic subclinical intramammary infections may be increased by the usage of both parenteral and intramammary drug application [2,4,5,6,7,8,9].

Beta-lactam antimicrobial agents are most widely used for the treatment of bovine mastitis. Despite the fact that we have at our disposal antibiotics such as oxacillin, amoxicillin/clavulanic acid, cephalosporins III and IV generation that inactivate β -lactamase, the resistance of staphylococcal isolates is achieved by biosynthesis of extracellular β -lactamase.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are defined as staphylococci resistant to methicillin and essentially all other beta-lactam antibiotics. Following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1990) antibiotic susceptibility testing to oxacillin, methicillin, nafcillin or cephalothin by the disk-diffusion tests can be used for the identification of MRSA [7].

Comparative studies of three standard methods (broth dilution, agar dilution, disk diffusion) for determination of resistance to cloxacillin and other penicillinase-resistant antibiotics showed a 100% agreement in methods [10].

The genome of *Staphylococcus aureus* is well known, as well as the significance of some genes, such as the *mecA* gene which encodes resistance to all beta-lactam antibiotics and *bla-Z* gene which encodes resistance to penicillin [1]. In Korea, among 835 *Staphylococcus aureus* strains isolated from bovine mastitic milk samples,

the *mecA* gen was found in 13 strains and defined as MRSA [11]. In Hungary, 375 *Staphylococcus aureus* strains were isolated and *mecA* gene was present in 28 strains [2]. However, the *mecA* gene was not found among 811 *Staphylococcus aureus* strains isolated from bovine mastitis in 11 countries in Europe and in the United States [12]. In Finland also, the *mecA* gene was not found in 647 strains isolated from mastitic milk samples [1].

Since the successful treatment depends on the choice of antimicrobials, we have decided to present the results of antibiograms and sensitivity of *Staphylococcus aureus* isolates that were routinely tested during the period of five years because of their practical and clinical applicability.

MATERIALS AND METHODS

Samples

Susceptibility of *Staphylococcus aureus* strains to different antimicrobial agents was analyzed on milk samples taken from two groups. The first group includes the samples that were collected in 2002 from 16 herds comprised of 18 to 37 cows within the National Programme of Udder Health Monitoring (2002). The samples taken from the second group in the years 2007, 2008, 2009, and 2010 were collected and delivered by the veterinarians following the instructions of the Veterinary Administration.

A total of 45,000 milk samples from bovine intramammary clinical and subclinical infections were used in this study.

Microbiological procedures

Bacterial species were identified by the microbiological procedures as described by Quinn et al. [7]. Samples collected from individual quarters of the udder were cultured onto Columbia agar plates containing 5% of defibrinated ovine blood („Merck“), incubated aerobically for 18-24 h at 37 °C.

Identification of bacterial isolates was made on the basis of colony morphology, catalase and coagulase tests, a tube coagulase test and by the detection of clumping factor with rabbit plasma on microscope slides, following the „Merck“ protocol.

All strains were identified as *Staphylococcus aureus* according to the results of the positive coagulase test. Additionally, from the strains isolated in 2002, the API ID 32 Staph system („bioMerieux“) was used for the species identification, and also DNase test was used on the first group of samples. Totally, 2719 strains identified as *Staphylococcus aureus* were routinely tested to some different antimicrobial agent by the agar-disc diffusion technique on Mueller-Hinton agar (Bio-Rad) and Oxoid test disc. As positive control *Staphylococcus aureus* ATCC 29213 standard strain was used.

RESULTS AND DISCUSSION

The number of the tested isolates of *Staphylococcus aureus* within the period of five years and the results for susceptibility testing of antimicrobial agents are presented in Table 1.

In the results presented in Table 1, 70.77% of strains isolated in 2002 were penicillin-susceptible, higher than any other year, after that 39.13% of strains in 2007,

Table 1. The number of *Staphylococcus aureus* strains and results for susceptibility testing to antimicrobial agents

Year	No. of strains		P 6 µg	AMC	OX 5 µg	CFX 30 µg	CFP 75 µg	L	SXT	ENF 5 µg	OTC	N	GM	KAN
2002	130	S	92	122			130	89	48		118	102		
		%	70.77	93.85			100	68.46	36.92		90.77	78.46		
2007	69	S	27	66	46	28	69	38		64		37	44	42
		%	39.13	95.65	66.66	40.58	100	55.07		92.75		53.62	63.77	60.87
2008	1364	S	512	1281	920	500	1239	735		1197		578	761	437
		%	37.54	93.91	67.45	36.66	90.83	53.88		87.76		42.37	55.79	32.0
2009	885	S	336	846	633	360	806	578		520		48		
		%	37.96	95.59	71.52	40.67	91.07	65.31		58.76		5.42		
2010	271	S	121	256	240	233	260	126	48	185	10	32		3
		%	44.65	94.46	88.56	85.98	95.94	46.49	17.71	68.26	3.69	11.81		1.11
Σ=	2719													

S=susceptible; P=penicillin; AMC=amoxicillin+clavulanic acid; OX=oxacillin; CFX=cefalexin; CFP=cefoperazone; L=lincomycin; SXT=trimethoprim+sulfamethoxazole; ENF=enrofloxacin; OTC=oksitetraciklin; N=neomycin; GM=gentamicin; KAN=kanamicin

37.54% in 2008, 37.96% in 2009, and 44.65% in 2010. 66.66% of oxacillin-susceptible strains were found in 2007, 67.45% in 2008, 71.52% in 2009 and 88.56% in 2010.

For potentiated penicillins like amoxicillin and clavulanic acid 93.85% to 95.65% of susceptible strains were found. For first-generation cephalosporins 36.66% - 85.98% of strains demonstrated susceptibility, while for third-generation cephalosporins 100% of strains were susceptible in 2002 and 2007, but 91.07% of isolated strains were susceptible in 2009 and 95.94% in 2010.

Staphylococcus aureus investigated strains showed different susceptibility against antimicrobial agents: tetracyclines, lincosamides, aminoglycosides, fluoroquinolones and sulfonamides. The majority of authors have presented different results, but some agreement has also been found.

In Finland [1] penicillin resistant *Staphylococcus aureus* strains were isolated in the range from 32.1% to 49.3%. In Turkey [13], resistance to penicillin was detected in 62.5% strains, to amoxycillin/clavulanic acid in 2.9%, to cloxacillin in 22.1%, to neomycin in 30.9%, oxytetracycline in 31.6%, to trimethoprim/sulphamethoxazole in 37.5%.

In Croatia [3], 88% of strains were resistant to penicillin, 81% to ampicillin, only 4% to cloxacillin and resistance was not detected to cefoperazone and amoxicillin/clavulanic acid. In several different countries of Europe and in the United States [12] among 811 *Staphylococcus aureus* strains, 5% to 51% (on average 36.4%) were resistant to beta-lactamase antimicrobial agents. In Argentina, [14] penicillin resistance was detected in 40.3% of *Staphylococcus aureus* isolates.

It can be seen that our results are in accordance with the results reported in other laboratories. Resistant strains are present in almost all dairy cow herds and around 50% of *Staphylococcus aureus* isolates are penicillin-resistant, but they showed different susceptibility to other antimicrobials.

CONCLUSION

Staphylococcus aureus isolates from mastitic cows in Croatia are resistant to β -lactam antibiotics, aminoglycosides, lincosamides, oxytetracycline, fluoroquinolones and sulfonamides. The data on antimicrobial susceptibility can help determine the choice of empirical initial treatment.

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Sažetak

Osjetljivost na antimikrobne tvari sojeva bakterije *Staphylococcus aureus* izdvojenih iz mlijeka krava s upalom vimena

Prikazani su rezultati rutinskih mikrobioloških nalaza i osjetljivost sojeva vrste *Staphylococcus aureus* izdvojenih iz mlijeka krava s različitim oblicima upala mliječne žlijezde tijekom pet godina. Ukupno je 2.719 sojeva vrste *Staphylococcus aureus* bilo pretraženo na osjetljivost na 12 antibiotika disk-difuzijskim postupkom. Sojevi su bili izdvojeni iz 45.000 uzoraka sekreta vimena na Columbia agaru s 5% ovčje krvi (0,01 ml na četvrtinu hranjive podloge). Identificirani su bili prema morfološkim osobinama, tvorbi katalaze i koagulaze, a sojevi iz 2002. godine i API ID 32 STAPH sustavom. Postotak sojeva osjetljivih na penicilin bio je najviši 2002., kada je iznosio 70,77%, a zatim slijedi 2007. s 39,13%, 2008. s 37,54%, 2009. s 37,96% i 2010. godina s 44,65%. Oksacilin osjetljivih sojeva utvrđeno je 66,66% u 2007., 67,45% u 2008., 71,52% u 2009. te 88,56% u 2010. godini. Za amoksisicilin s klavulanskom kiselinom osjetljivost je iznosila 93,85% – 95,65% sojeva. Na cefalosporine I. generacije osjetljivo je bilo 36,66% – 85,98% sojeva, dok je na cefalosporin III. generacije bilo osjetljivo 100% sojeva iz 2002. i 2007. godine, a 91,07% 2009. i 95,94% izolata iz 2010. godine. Sojevi su pokazali različitu osjetljivost na tetracikline, linkozamide, aminoglikozide, fluorirane kinolone i sulfonamide. Rezultati upućuju na postojanje sojeva rezistentnih na sve skupine antibiotika.

Ključne riječi: *Staphylococcus aureus*; krava; mastitis; antimikrobni lijekovi

