Factors responsible for subclinical mastitis in cows caused by *Staphylococcus chromogenes* and its susceptibility to antibiotics based on *bap*, *fnbA*, *eno*, *mecA*, *tetK*, and *ermA* genes

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ABSTRACT

The aim of this study was to recognize selected factors of virulence determining the adhesion of *Staphylococcus chromogenes* to cows' udder tissues in subclinical mastitis and to evaluate the susceptibility of this pathogen to antibiotics. The subjects of the study were 38 isolates of *Staph. chromogenes* from 335 samples of milk from cows with subclinical coagulase-negative staphylococci mastitis. Somatic cell count ranged between 216,000 and 568,000/mL of milk (average 356,000/mL of milk). We confirmed the ability to produce slime in 24 isolates (63.2%), and the ability to produce protease in 29 isolates (76.3%). In each slime-producing isolate, the *bap* gene was not found, and the *fnbA* and *eno* genes were not detected. In vitro tests showed that ceftiofur had the highest effectiveness against *Staph. chromogenes* (89.5% of susceptible isolates). Minimum inhibitory concentrations ranged from 0.06 to 2 µg/mL for susceptible isolates. The minimum concentrations required to inhibit growth of 90 and 50% of the isolates for ceftiofur were at or below the cutoffs recommended by the Clinical and Laboratory Standards Institute (2 and 0.06 µg/mL, respectively). A significant percentage of the isolates were susceptible to other β-lactam antibiotics: amoxicillin with clavulanic acid (84.2%) and ampicillin (81.6%). The lowest effectiveness among β-lactams was for penicillin (73.7% of susceptible isolates), and the minimum inhibitory concentration for penicillin ranged from <0.06 to 8 µg/mL. None of the examined isolates had the *mecA* gene, but β-lactamase was detected in 4 isolates (10.5%). Erythromycin and oxytetracycline exhibited the lowest activity against *Staph. chromogenes*

(71.1 and 63.2% of susceptible isolates, respectively). The genes *tetK* (6 isolates) and *ermA* (1 isolate) were also detected.

Key words: *Staphylococcus chromogenes*, coagulase-negative staphylococci, subclinical mastitis

INTRODUCTION

Mastitis is one of the most common diseases in dairy cows, and its occurrence is connected with substantial economic losses (Pyörälä and Taponen, 2009; Frey et al., 2013; Tremblay et al., 2013; Tomazi et al., 2015). Each type of mastitis results in decreases in milk production and quality. The milk becomes unsuitable for human consumption and for technological processes. Bacterial mastitis is caused by several pathogens, but the most frequently isolated bacterial species are CNS (Pyörälä and Taponen, 2009).

Widely found in the natural environment, CNS colonize both animal and human skin and mucous membranes. For many years, CNS were thought to be nonpathogenic, but they have now become known as the predominant etiological factor in mastitis in cows in numerous countries (Makovec and Ruegg, 2003; Pitkälä et al., 2004; Rajala-Schultz et al., 2004; Taponen et al., 2007). More than 15 strains of CNS are known and have been identified as etiological factors in mastitis (Thorberg et al., 2009; Waller et al., 2011; Tomazi et al., 2015). Based on the literature (Tomazi et al., 2015) and one of our previous studies (Bochniarz et al., 2013), we can infer that *Staph. chromogenes* is one of the most common CNS species in mastitis. It is interesting to explore what mechanisms enable this pathogen, which appears in saprophytic form in the environment, to overcome the physical barrier of the teat canal, adhere to cells, and form biofilm, which protects it against removal from the udder during milking.

The initial stages of infection involve *Staphylococcus* spp. proteins that are responsible for adherence to the

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host extracellular matrix (described as microbial surface components recognizing adhesive matrix molecules, MSCRAMM) and other surface proteins that promote biofilm formation or resistance to opsonophagocytosis (Cree et al., 1994). *Staphylococcus* spp. proteins have the ability to bind to the components of extracellular matrix—laminin and fibronectin, among others (Paulsson et al., 1992). Laminin is a component of the foundation membrane between the epithelial cells and connective tissue in the mammary gland. Fibronectin appears in 2 forms: soluble plasma fibronectin, which occurs in various bodily fluids, and insoluble cellular fibronectin, which is combined with the cells of different tissues. Owing to the biological functions of fibronectin (facilitation of phagocytosis, contributions to the reconstruction of damaged tissues, regulation of cytoskeleton organization), it becomes a target molecule for the bacteria. Fibronectin-binding protein is encoded by the *fnbA* gene, and laminin-binding protein is encoded by the *eno* gene (Simojoki et al., 2012).

Invasiveness of pathogens is also determined by their ability to proliferate on the surface where they adhere (Melchior et al., 2011). During this process, bacterial biofilm is formed out of the cellular matrix and daughter cells adherent to it, bound together by slime produced by the bacteria. This substance is described as amorphous and sticky, loosely attached to the cell wall of microorganisms (Matthews et al., 1991). The polysaccharide molecule (molecule weight 100,000 kDa) is a main component of slime. It is largely made up of glucose and *N*-acetyl-glucosamines, and it is resistant to enzymes produced by the host organism. Biofilm forms a protective barrier against phagocytosis, inhibits opsonization by the antibodies, and hinders the access of antibiotics (Costerton et al., 1999; Melchior et al., 2006). Biofilm formation plays an important role in virulence, but its role as a virulence factor in *Staph. chromogenes* has been poorly studied (Tremblay et al., 2013).

Staphylococcus survival in the udder depends on the availability of iron in its free form, Fe^{3+} . In the inflammatory process, a significant increase in levels of iron-binding proteins (lactoferrin and transferrin) in milk can be observed. These substances hinder the availability of iron to bacteria (Chaneton et al., 2008). The ability to produce protease, which allows the pathogens to access the iron that is vital to their survival, is one of the most essential elements of their pathogenicity.

The aim of this study was to identify the factors that determined the adhesion and survival of *Staph. chromogenes* isolates in the udders of cows with subclinical mastitis, and to evaluate the isolates' susceptibility to antibiotics.

MATERIALS AND METHODS

The subject of the study were 38 isolates of *Staph. chromogenes* taken from 335 samples of milk from cows with subclinical mastitis caused by CNS. These isolates originated from the milk of 27 cows from farms in the Lubelskie region of Poland. In 16 cows, *Staph. chromogenes* was isolated from only 1 quarter, and in 11 cows it was isolated from 2 quarters. Cows with *Staph. chromogenes* were from 5 herds that used various housing systems (4 freestall or loose and 14 tiestall systems). Clinical examination of cows and macroscopic evaluation of the milk were performed before milk samples were collected for bacteriological testing. Cows received no medications during the ongoing lactation.

Milk was bacteriologically tested according to generally accepted procedures. Milk samples were brought to room temperature and then thoroughly mixed and cultured on agar medium (BTL, Łódź, Poland) supplemented with sterile, defibrinated sheep blood (5% of the agar solution volume). After incubation for 24 h at 37°C in aerobic conditions, pathogens were initially identified based on colony morphology and gram-stained using microscopic specimens.

Identification of CNS species was performed using a commercial API Staph test (bioMérieux, Marcy-l'Étoile, France) based on determinations of 19 biochemical features. The procedure followed the manufacturer's recommendations (<http://biomerieux-usa.com/clinical/api>).

Mastitis classification was conducted according to generally accepted rules (Gentilini et al., 2002; De Vliegher et al., 2003; Moon et al., 2007), which include clinical examination of the cows, milk bacteriological testing, and SCC in milk samples (Fossomatic; Foss, Hillerød, Denmark). An SCC >200,000/mL of milk and the presence of bacteria in milk (despite a lack of general symptoms in the cow) was considered to be subclinical mastitis.

Evaluation of Capacity to Form Slime

Slime-producing capacity was determined using Congo red agar (Sigma, St. Louis, MO; Freeman et al., 1989). Overnight cultures in TSB (tryptic soy broth) were inoculated onto Congo red agar plates and incubated at 37°C for 24 h. Biofilm formation was detected based on the presence of black or almost black colonies on the agar.

Evaluation of Capacity to Form Protease

A base solution of nutrient gelatin (Oxoid, Basingstoke, UK) with 128 g concentration in 1 L of distilled

water was sterilized at 121°C for 15 min and poured onto Petri plates (9–10 cm diameter). After the medium congealed, the examined isolates were inoculated in a thin line. The plates with the medium were incubated at 37°C for 24 h. An area 4 times wider than the tested strain growth line was considered a positive result.

Evaluation of Capacity of Methicillin-Resistant CNS to Produce β -Lactamase

Determination of the CNS ability to produce β -lactamase was carried out using the β -Lactamase Test (Oxoid). The nitrocefin-impregnated tip of a stick was immersed in the colony of CNS strains on the agar medium, and a small number of bacterial cells was collected. The results were read after 5 min, and a stick color change to pink-red was considered positive.

Molecular Study

DNA was extracted from bacterial cells using the Genomic Mini kit (A&A Biotechnology, Gdansk, Poland) according to the manufacturer's instructions (http://aabiotech/home.pl/aabiotech/PDF/Genomic%20Mini_PL.pdf).

Real-time PCR was carried out using the Corbett apparatus. Pairs of primers specific to the *mecA*, *tetK*, *ermA*, *bap*, *fnbA*, and *eno* genes were used (Table 1). The PCR was carried out in real time using SYBR Green 1 dye, in thin-walled test tubes with a capacity of 100 μ L. The DyNAmo HS SYBR Green qPCR Kit (Finnzymes, Vantaa, Poland) was used, to conduct a high-specificity reaction.

The 20- μ L reaction mixture consisted of the following components: 2 μ L of the DNA matrix, 7.2 μ L of water, 0.4 μ L of each primer (final concentration 50 pM), and 10 μ L of Master Mix containing a hot start version of the modified polymerase Tbr (*Thermus brockianus*), a

buffer for the polymerase Tbr, dNTP, $MgCl_2$, and the intercalating SYBR Green 1 dye.

We evaluated the susceptibility of microorganisms to antibiotics by determining antibiotic MIC according to the method of double dilution in a liquid base of Mueller-Hinton broth (CM0405; Oxoid) using flat-bottomed 96-well microplates. We examined *Staph. chromogenes* for its susceptibility to amoxicillin with clavulanic acid (Fluka, Buchs, Switzerland), ampicillin (Roth, Karlsruhe, Germany), ceftiofur (Fluka), lincomycin (Roth), erythromycin (Roth), penicillin (Roth), and oxytetracycline (Roth). Antibiotic concentrations ranged between 0.06 and 32 μ g/mL. Initial antibiotic solutions were diluted 1:100 in Mueller-Hinton bouillon; then, 100 μ L of each operating solution was added to the first holes of titration plates and transferred to the next. The final concentration of the antibiotic in each dent was 2 times lower than in the previous one. Bacterial colonies collected from the agar plates were suspended in 4 mL of 0.85% NaCl solution until a density of 0.5 on the McFarland scale was reached.

RESULTS

Staph. chromogenes made up 11.3% of all CNS isolated from cows with subclinical mastitis. No macroscopic changes in milk or symptoms in the cows were found, and SCC ranged between 216,000 and 568,000/mL of milk (average 356,000/mL of milk).

Phenotypic factors responsible for the pathogenicity of *Staph. chromogenes* in isolate from subclinical mastitis are presented in Table 2. The capacity to produce slime was confirmed in 24 isolates (63.2%); however, non-slime-producing isolates exhibited the *bap* gene. Fibronectin-binding and laminin-binding proteins were not detected. The *bap* and *eno* genes were also not detected in reference strains, but the *fnbA* gene was detected in *Staphylococcus epidermidis* ATCC 12228

Table 1. Primers for PCR

Gene	Protein	Primer sequence (5–3)	Amplicon length, bp
<i>mecA</i> ¹		Forward: AAAATCGATGGTAAAGGTTGGC Reverse: AGTTCCTGCAGTACCGGATTTGE	533
<i>tetK</i> ¹		Forward: GTAGCGACAATAGGTAATAGT Reverse: GTAGTGACAATAAACCTCCTA	360
<i>ermA</i> ¹		Forward: GTTCAAGAACAATCAATACAGAG Reverse: GGATCAGGAAAAGGACATTTTAC	421
<i>bap</i> ²	Biofilm-associated protein	CCCTATATCGAAGGTGTAGAATT GCTGTTGAAGTTAATACTGTACCTGC	971
<i>fnbA</i> ²	Fibronectin-binding protein A	GTGAAGTTTTAGAAGGTGGAAAGATTAG GCTCTTGTAAGACCATTTTCTTCAC	643
<i>eno</i> ²	Laminin-binding protein	ACGTGCAGCAGCTGACT CAACAGCATCTTCAGTACCTTC	302

¹Used according to Kot et al. (2012).

²Used according to Simojoki et al. (2012).

Table 2. Factors of virulence among isolates of *Staphylococcus chromogenes* from subclinical mastitis in cows

Factor of virulence	<i>Staphylococcus chromogenes</i> (n = 38)	
	No.	%
Production of slime	24	63.2
Production of protease	29	76.3
β -Lactamase	4	10.5

(Table 3). The capacity to produce protease was observed in 29 isolates of *Staph. chromogenes* (76.3%).

Table 4 shows the MIC for 6 antimicrobial agents against 38 isolates of *Staph. chromogenes* isolated from subclinical mastitis in cows. The β -lactams (amoxicillin, ampicillin, and cephalosporins) are widely used to treat bovine mastitis, and we observed these antibiotics as highly effective. Ceftiofur showed the highest activity (89.5% of isolates were susceptible; Table 5), and ceftiofur MIC ranged from 0.06 to 2 $\mu\text{g/mL}$ for the susceptible isolates. The minimum concentrations required to inhibit growth of 90% (MIC₉₀) and 50% (MIC₅₀) of the isolates for ceftiofur were at or lower than the cutoffs recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013; 2 and 0.06 $\mu\text{g/mL}$, respectively). Only 4 isolates (10.5%) were resistant to ceftiofur. A substantial percentage of the isolates were susceptible to amoxicillin with clavulanic acid (84.2%) and to ampicillin (81.6%). The MIC for both amoxicillin and ampicillin were located in the confidence intervals of <0.06 to >32 $\mu\text{g/mL}$, but a difference was recorded in MIC₉₀ and MIC₅₀ levels for these antibiotics. Growth of 50% of tested isolates was inhibited by 0.06 $\mu\text{g/mL}$ amoxicillin and 0.125 $\mu\text{g/mL}$ ampicillin. However, to inhibit growth of 90% of tested isolates, 1 $\mu\text{g/mL}$ amoxicillin was needed but only 0.5 $\mu\text{g/mL}$ ampicillin. The lowest effectiveness among β -lactam antibiotics was observed for penicillin (73.7% susceptible isolates). The *mecA* gene was undetected, but β -lactamase was observed in 4 isolates (10.5%). The remaining antibiotics were far less effective. Only

71.1% of *Staph. chromogenes* isolates were susceptible to erythromycin, and barely 1 of 11 isolates, which were resistant to erythromycin, had the *ermA* gene. The lowest activity in vitro was observed for oxytetracycline (63.2% of isolates were susceptible). The *tetK* gene was detected in 6 of 14 isolates, which exhibited phenotypic susceptibility to oxytetracycline.

DISCUSSION

The CNS play substantial role as etiological factors in mastitis in cows, and *Staph. chromogenes* is one of the most commonly isolated species of CNS (Taponen et al., 2006; Lthje and Schwarz, 2006; Sawant et al., 2009; Frey et al., 2013; Tomazi et al., 2015).

Rajala-Schultz et al. (2004), who studied milk samples from cows affected by mastitis in the United States from 2001 to 2002, identified 158 CNS species, with the highest percentage being *Staph. chromogenes*. A dozen or so years earlier, a study conducted by Matthews et al. (1991) also demonstrated that the most prevalent species of CNS obtained from milk was *Staph. chromogenes*. In a previous study (Bochniarz et al., 2013), we found that *Staph. chromogenes* was the most frequently isolated species of CNS in subclinical mastitis. This species has also been isolated in heifers with mastitis (De Vlieghe et al., 2003; Sawant et al., 2009).

Similar to other species of CNS, *Staph. chromogenes* is among the physiological bacterial flora that exist on the skin of mammary glands. In the literature, it has been widely shown how this pathogen colonizes the skin and apexes of the teats. White et al. (1989) isolated *Staph. chromogenes* from 62% of samples from teat skin, and Trinidad et al. (1990) recorded the same species in 43% of samples from the teat apexes. In a study by De Vlieghe et al. (2003), *Staph. chromogenes* was prevalent among CNS isolated from the teat canals of both heifers and cows in the dry period and postpartum.

Staphylococcus chromogenes most often causes barely noticeable subclinical mastitis (Bochniarz et al., 2013). The early diagnosis and treatment of mastitis leads to

Table 3. Distribution of genes among isolates of *Staphylococcus chromogenes* from subclinical mastitis in cows, and control reference strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*

Gene	<i>Staphylococcus chromogenes</i> (n = 38)		<i>Staph. aureus</i> (ATCC 43300)	<i>Staph. aureus</i> (ATCC 29213)	<i>Staph. epidermidis</i> (ATCC 12228)
	No.	%			
<i>mecA</i>	0	0.0	+	—	—
<i>tetK</i>	6	15.8	—	+	+
<i>ermA</i>	1	2.6	+	—	—
<i>bap</i>	0	0.0	—	—	—
<i>fnbA</i>	1	2.6	—	—	+
<i>eno</i>	0	0.0	—	—	—

Table 4. Susceptibility levels ($\mu\text{g/mL}$) among isolates ($n = 38$) of *Staphylococcus chromogenes* from subclinical mastitis in cows

Antibiotic	MIC ₉₀ ¹	>32	32	16	8	4	2	1	0.5	0.25	0.125	0.06	<0.06	MIC ₅₀ ¹
Amoxicillin with clavulanic acid	1	1	1				2	2	1	5	7	9	10	0.06
Ampicillin	0.5	1	1				1	1	3	6	10	5	10	0.125
Oxytetracycline	>32	6	6	2				2	2	2	10	6		0.5
Ceftiofur	2				4		1	3	7	14	8	1		0.25
Penicillin	2				1	1	3	0	1	4	6	8	14	0.06
Erythromycin	16	2	2	1	4	2		5		1	11	8	2	0.125

¹MIC₉₀ and MIC₅₀ are the minimum concentrations required to inhibit growth of 90 and 50% of the isolates tested, respectively.

quicker elimination of infection. In some cases, mastitis caused by *Staph. chromogenes* has resolved on its own. Aarestrup and Jensen (1997) found that infection of the udder caused by *Staph. chromogenes* was observed shortly after delivery and disappeared quickly. In contrast, infection caused by other species of CNS lasted for long periods or recurred during the same lactation. In a study carried out by Taponen et al. (2007), persistent CNS infection in the mammary gland was observed in 46% of tested quarters and transient infection in 54%; *Staph. chromogenes* was the dominant species in both transient and persistent infections. Therefore, from the studies, we can infer that this pathogen can survive in the udder regardless of desquamating epithelium and the flow of milk during milking.

Adhesion of microorganisms to host tissues is the first stage of pathogenesis in udder infections (Aguilar et al., 2001). In this process, *Staphylococcus* spp. proteins, such as laminin-binding protein and fibronectin-binding protein, are vital because they allow the pathogen to bind to the host's extracellular matrix (Cree et al., 1994). The next stage, which simplifies the survival of pathogens in the udder, is the creation of bacterial biofilm. Biofilms are structured communities of bacterial cells enclosed in a self-produced matrix and attached to surfaces (Costerton et al., 1999). Biofilm protects the bacterial cells against the host's immunological defenses and against the activity of antibiotics used to treat mastitis (Costerton et al., 1999; Melchior et al., 2006). In vitro tests indicate that *Staphylococcus* spp. that reside within biofilm are 10 to 1,000 times

more resistant to antibiotics than plankton cells of the same strains. Biofilm formation in *Staphylococcus* spp. is associated with several factors, including biofilm-associated protein (Cucarella et al., 2004). The results of our study did not confirm the presence of both the *bap* gene and the *fmbA* and *eno* genes in tested isolates. However, an important role in adhesion may be played by the mucus capsule in *Staphylococcus* spp., which enables bacterial adhesion and the production of bacterial biofilm (Aguilar et al., 2001; Lee and Lee, 2006). In the present study, we observed the ability to produce slime, facilitating adhesion of bacterial cells, in more than 60% *Staph. chromogenes* isolates. Simojoki et al. (2012) observed slime production in Congo red agar analysis with glucose supplementation in 14.1% isolates of CNS from mastitis, although *Staph. chromogenes* were less common than other species (only 1/55 isolates was slime-positive). Similar to our findings, the *bap* gene was not detected any of the 84 CNS isolates originating from mastitis Simojoki et al. (2012).

Protease also plays a significant role in the survival of *Staphylococcus* spp. in the udder, because of its ability to bind hemoglobin, lactoferrin, and transferrin in the place of iron binding. In this way, iron is detached and is more accessible to pathogen cells (Lacasse et al., 2008; Chaneton et al., 2008). Staphylococci require higher concentrations of iron for formation of biofilm than for vegetative growth (Weinberg, 2004). In the present study, the capacity to form protease was observed in 76.3% of isolates.

We observed the highest effectiveness against *Staph. chromogenes* isolates for cephalosporins, amoxicillin, and ampicillin. The lowest percentage of resistant isolates was observed for ceftiofur (10.5%), which was the only antibiotic for which the MIC₉₀ and MIC₅₀ were at or below the cutoffs recommended by CLSI (2013). Lthje and Schwarz (2006) recorded even higher rates of effectiveness for ceftiofur: only 0.3% of CNS isolates were resistant. In the present study, the β -lactam antibiotic with the lowest activity was penicillin. Methicillin resistance, determined by the *mecA* gene, was not found. Watts and Owens (1989) also demonstrated that amoxicillin with clavulanic acid was highly effective

Table 5. Antimicrobial susceptibility among isolates ($n = 38$) of *Staphylococcus chromogenes*

Antibiotic	Sensitive, no. (%)	Resistant, no. (%)
Amoxicillin with clavulanic acid	32 (84.2)	6 (15.8)
Ampicillin	31 (81.6)	7 (18.4)
Oxytetracycline	24 (63.2)	14 (36.8)
Ceftiofur	34 (89.5)	4 (10.5)
Penicillin	28 (73.7)	10 (26.3)
Erythromycin	27 (71.1)	11 (28.9)

tive (100% susceptible isolates) but that penicillin had lower effectiveness. Lüthje and Schwarz (2006) found that among CNS strains, including those dominated by *Staph. chromogenes*, there were no isolates resistant to penicillin, and the MIC₉₀ and MIC₅₀ for penicillin was at ≤ 0.006 ng/mL and 0.12 ng/mL, respectively. These values were lower than the cutoffs for penicillin recommended by the CLSI (2013). In a study conducted by Salmon et al. (1998), the MIC₉₀ value for penicillin against CNS was 0.25 ng/mL. In contrast, the MIC₉₀ for penicillin in a study by Gentilini et al. (2002) was 4 ng/mL, and in a study by Thorberg et al. (2009), it was 32 ng/mL. These significant differences in MIC result from the fact that various CNS are often regarded as a single group. It is advisable to evaluate CNS species individually, because they can exhibit different susceptibility patterns (Sawant et al., 2009).

The literature indicates that among antibiotics used to treat bovine mastitis, erythromycin showed low activity against CNS (Lüthje and Schwarz, 2006; Piessens et al., 2012). In the present study, although a significant percentage of isolates were resistant to erythromycin (28.9%) and tetracycline (38.7%), the *ermA* gene was detected only in 1 isolate, and the *tetK* gene was found in 6 isolates. Contrary to the present study, Devriese et al. (2002) did not record resistance to erythromycin, and only 2/70 isolates of *Staph. chromogenes* were resistant to oxytetracycline. On the other hand, in Frey et al. (2013) found that a significant percentage of CNS isolates obtained from bovine milk were more often resistant to penicillin than to erythromycin or tetracycline (23.3, 7.0, and 15.8%, respectively). Similar to the present study, the *mecA* gene was not detected, regardless of isolates' resistance to penicillin.

CONCLUSIONS

To cause mastitis, pathogens must possess the ability to adhere and survive in cows' udders. In our study, tested isolates of *Staph. chromogenes* did not have the gene encoding biofilm-associated protein (*bap*) or the genes encoding proteins responsible for binding with extracellular matrix (*fmbA* and *eno*). However, a significant percentage of isolates produced the slime needed in biofilm formation. We also observed that a high percentage of tested isolates could form protease, which facilitates access to the iron necessary for the growth of *Staphylococcus* spp. We also found that most of the β -lactam antibiotics were highly effective against *Staph. chromogenes*. The results of the study were satisfactory, because cephalosporins, amoxicillin with clavulanic acid, and ampicillin are among the antibiotics most commonly used to treat mastitis in cows.

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