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Efficacy of Targeted 5-day Combined Parenteral and Intramammary Treatment of Clinical Mastitis Caused by Penicillin-Susceptible or Penicillin-Resistant *Staphylococcus aureus*

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Taponen S, Jantunen A, Pyörälä E, Pyörälä S: Efficacy of targeted 5-day combined parenteral and intramammary treatment of clinical mastitis caused by penicillin-susceptible or penicillin-resistant *Staphylococcus aureus.* Acta vet. scand. 2003, 44, 53-62. – Combined parenteral and intramammary treatment of mastitis caused by *Staphylococcus aureus* was compared to parenteral treatment only. Cows with clinical mastitis (166 mastitic quarters) caused by *S. aureus* treated by veterinarians of the Ambulatory Clinic of the Faculty of Veterinary Medicine during routine farm calls were included. Treatment was based on in vitro susceptibility testing of the bacterial isolate. Procaine penicillin G (86 cases due to *ß*-lactamase negative strains) or amoxycillin-clavulanic acid (24 cases due to *ß*-lactamase positive strains) was administered parenterally and intramammarily for 5 days. Efficacy of treatments was assessed 2 and 4 weeks later by physical examination, bacteriological culture, determination of CMT, somatic cell count and NAGase activity in milk. Quarters with growth of *S. aureus* in at least one post-treatment sample were classified as non-cured. As controls we used 41 clinical mastitis cases caused by penicillin-susceptible *S. aureus* isolates treated with procaine penicillin G parenterally for 5 days and 15 cases due to penicillin-resistant isolates treated with spiramycin parenterally for 5 days from the same practice area. Bacteriological cure rate after the combination treatment was 75.6% for quarters infected with penicillin-susceptible *S. aureus* isolates, and 29.2% for quarters infected with penicillin-resistant isolates. Cure rate for quarters treated only parenterally with procaine penicillin G was 56.1% and that for quarters treated with spiramycin 33.3%. The difference in cure rates between mastitis due to penicillin-susceptible and penicillin-resistant *S. aureus* was highly significant. Combined treatment was superior over systemic treatment only in the *ß*-lactamase negative group.

cow; mastitis; ß-lactamase.

Introduction

In recent years, the proportion of *Staphylococcus aureus* as a mastitis causing agent has decreased in many countries, including Finland (*Myllys et al.* 1998). However, *Staphylococcus aureus* still remains the most harmful udder pathogen, since the disease responds poorly to antimicrobial treatment and often remains chronic. Quarters affected with chronic *S. aureus* mastitis may shed large amounts of bacteria, increase the risk of other cows in the herd becoming infected, and raise bulk milk somatic cell count. In Finland and other Nordic countries, targeted treatment of bovine mastitis is strongly recommended (*Anonymous* 1996,

Anonymous 1998). Milk samples for bacteriological examination should be taken from the affected quarter and the antimicrobial treatment should be based on the bacteriological diagnosis. The in vitro susceptibility of the bacterial isolates should be determined as appropriate; *ß*-lactamase test is recommended for *S. aureus* isolates, as a large proportion of them are resistant to penicillin (*Aarestrup & Jensen* 1998, *Myllys et al.* 1998). Systemic or a combination of systemic and intramammary treatment has been suggested to be preferable in clinical *S. aureus* mastitis, due to better penetration of the drug into the inflamed mammary tissue (*Ziv* 1980, *Franklin et al.* 1986, *Sandholm et al.* 1990, *Prescott et al.* 2000). Studies on the efficacy of systemic treatment of staphylococcal mastitis are not abundant (*Funke* 1982, *Jarp et al.* 1989, *Pyörälä & Pyörälä* 1998), and even less has been published on the effect of combination treatment (*Owens et al.* 1988, *Perner et al.* 2002). In many mastitis studies, no attention has been paid to the in vitro susceptibility of the causing agent.

The aim of this study was to determine the effect of a 5-day targeted combination treatment on clinical *S. aureus* mastitis caused by *ß*-lactamase negative or positive isolates.

Materials and methods

The materials were collected in the practice area of the Ambulatory Clinic of the Faculty of Veterinary Medicine between years 1989 and 1997 and consisted of 166 quarter cases of clinical mastitis caused by *S. aureus* in 118 cows from 72 commercial dairy farms. The majority of the cows was of the Finnish Ayrshire breed. The median age of the cows was 4 years (third lactation), and 21% of the cows were in their first lactation. All cases of clinical mastitis caused by *S. aureus* and meeting the inclusion criteria were taken to the study materials. The inclusion criteria were as follows: antimicrobial treatment according to the study design and no concomitant systemic disease, teat lesions or chronic mastitis, i.e. mastitis that had persisted during the dry period or had been treated at least 2 times during the same lactation or had caused elevated somatic cell count for a long period, i.e. months. The first part of the study material originates from a larger field trial where the effects of different cow factors and duration of treatment were investigated (*Pyörälä & Pyörälä* 1998). From that material, 56 cases of clinical mastitis caused by *S. aureus* and treated for 5 days were included in the present study. The second part of the material was collected after the first part by the same veterinarians on the same practice area. In brief, the farmers contacted the veterinarians of the Ambulatory Clinic when they detected a case of mastitis in the herd. The veterinarian visited the farm, examined the cow and estimated milk somatic cell count (SCC) using the California Mastitis Test (CMT). The history of the cow (identity, age, stage of lactation etc.) was recorded. Local and systemic clinical signs, including rectal temperature, and milk appearance were observed and recorded on a form. Using the notes on the forms the signs were later scored from 1 to 3, where 1 = clots and flakes seen in the milk but no other signs, 2 = body temperature 39.0-40.5 °C and/or slight anorexia/depression, swelling and/or tenderness in the affected quarter and moderate changes in milk appearance, and 3 = body temperature >40.5 °C and/or severe anorexia and depression and/or recumbent, severe swelling, firmness and soreness in the quarter and severe changes in the milk appearance. For statistical analyses, scores 2 and 3 were grouped together: 1 = mild signs and 2 & 3 = moderate/severe signs. The veterinarian took an aseptic milk sample from the affected quarter(s) for bacteriological examination and N-acetyl-*ß*-D-glucosaminidase (NAGase) activity determination.

In severe cases, the veterinarian started the treatment immediately with penicillin G, if there was no reason, based on history of the herd, to suspect that mastitis was caused by *ß*-lactamase positive *S. aureus.* In milder cases (sign score 1), the treatment was started after having obtained the bacterial diagnosis and the result of the *ß*-lactamase test, which usually took 2 additional days.

Milk samples were cultured for bacteriological diagnosis in the laboratory of the Ambulatory Clinic by use of routine methods (*Honkanen-Buzalski & Seuna* 1995). The *S. aureus* isolates were divided into *ß*-lactamase positive and negative by use of a *ß*-lactamase test (*Myllys* 1995). A total of 127 isolates proved to be in vitro *ß*-lactamase negative, and 39 positive. *ß*-lactamase positive isolates were tested to be in vitro susceptible to the antibiotic used in the trial by an agar diffusion test (*Myllys* 1995). NAGase activity in the milk of the affected quarter taken on the day of diagnosis and at the follow-up visits was determined (*Pyörälä & Pyörälä* 1997) in the laboratory of the Ambulatory Clinic.

Cows with mastitis caused by *ß*-lactamase negative *S. aureus* were treated with procaine penicillin G (Ethacilin, Intervet International, The Netherlands; or Penovet, Boehringer Ingelheim Agrovet A/S, Denmark) parenterally at a dose of 20 mg/kg once a day for 5 days combined with intramammaries containing 500 mg penicillin and 300 mg neomycin once a day for 4 days (Vonapen, Intervet International, The Netherlands) (86 cases), or parenterally only at a dose of 20 mg/kg once a day for 5 days (41 cases). Cases caused by *ß*-lactamase positive *S. aureus* were treated with amoxycillin-clavulanic acid (Synulox, Pfizer Animal Health, UK) parenterally at a dose of 7.0 mg amoxycillin and 1.75 mg clavulanic acid per kg once a day for 5 days combined with intramammaries containing 200 mg amoxycillin, 50 mg clavulanic acid and 10 mg prednisolone once a day for 4 days (Synulox Lactating Cow, Pfizer) (24 cases) or parenterally only with spiramycin (Spiramycin, Rhône Mérieux, France) at a dose of 10 mg/kg once a day for 5 days (15 cases). The first injection of spiramycin was given intravenously by the veterinarian, the rest of the injections intramuscularly by the herd owner.

Efficacy of treatment was assessed twice by physical examination of the udder, bacteriological culturing and determination of SCC and NAGase activity in milk about 2 weeks (mean 14.9 days, min. 10 days, max. 23 days) and 4 weeks (mean 29.2 days, min. 26 days, max. 37 days) after the beginning of the treatment. SCC was determined in the laboratory of the Ambulatory Clinic using Coulter Counter method (*Tolle et al.* 1966). NAGase activity less than 40 U was classified as cured (*Pyörälä & Pyörälä* 1997). A quarter was classified as bacteriologically cured if growth of *S. aureus* was not detected in either of the post-treatment milk samples. For the groups with parenteral treatment alone, follow-up visits were made only once, about 4 weeks (mean 29.7 days, min. 20 days, max. 48 days) after the treatment, and SCC was not determined. In the comparisons between the treatment groups, only results from the post-treatment sample at 4 weeks after treatment were used.

Logistic regression analyses were used to test the effects of the following variables on the cure rates: penicillin susceptibility, treatments, parity (first or subsequent), stage of lactation (1-14 days post partum or >14 days post partum) and severity of mastitis on the day of diagnosis, measured by NAGase activity, CMT score or score of the clinical signs (mild or moderate/severe). Cows from the same farm and different quarters from the same cow were treated as if they were independent observations because the numbers of repeated observations of cows and quarters were small. The effect of the peni-

Table 1. Cure rates for different treatment groups with clinical mastitis caused by in vitro penicillin-susceptible or penicillin-resistant *S. aureus*. Cows were treated with parenteral administration alone or with concomitant parenteral and intramammary administration; duration of all treatments was 5 days. The efficacy of the treatment was assessed 2 and 4 weeks post-treatment for those treated with combined treatment and 4 weeks post-treatment for those treated parenterally only.

	Cure rates								
		Bacteriological				Milk NAGase[4]		Total[5]	
	Quarters	1 control[2]		2 controls[3]					
Treatment	n	n	%	n	%	n	%	n	%
Pen. G susceptible, total	127	91	71.7[a]			80	63.0[e]	71	55.9[i]
Systemic	41	23	56.1[c]			23	56.1	20	48.8
Systemic+IMM[1]	86	68	79.1[d]	65	75.6	57	66.3	51	59.3
Pen. G resistant, total	39	13	33.3[b]			13	33.3[f]	7	17.9[j]
Spiramycin systemic	15	5	33.3			8	53.3[g]	5	33.3
Amox.-clavul.systemic+IMM[1]	24	8	33.3	7	29.2	5	20.8[h]	2	8.3
Total	166	104	62.7			93	56.0	78	47.0

[1] IMM = intramammary administration
[2] *S. aureus* not detected 4 weeks post-treatment
[3] *S. aureus* not detected 2 and 4 weeks post-treatment
[4] NAGase <40 U 4 weeks post-treatment
[5] Total cure = clinical plus bacteriological cure and milk NAGase value <40 U 4 weeks post-treatment
Significance of difference: a-b: p<0.001, c-d: p=0.028, e-f: p=0.023, g-h: p=0.028, i-j: p<0.001.

cillin susceptibility on the cure rate was tested using a model where penicillin susceptibility and parity (first or subsequent) were included as factors. The effects of treatments were tested separately for the penicillin susceptible and resistant groups using a model where treatment and parity were included as factors. Variables that were not significantly related to the cure rate (likelihood ratio test) were dropped from the models. The difference between proportion of moderate and severe signs in early (first 14 days post partum) and later lactation was tested using the Chi-square test.

Results

Mastitis caused by *β*-lactamase negative *S. aureus* was found to have significantly higher cure rate than mastitis caused by *β*-lactamase positive *S. aureus* (p<0.001). The treatment (combination versus parenteral only) affected the bacteriological cure rate in the *β*-lactamase negative group significantly (p = 0.009), but not in the *β*-lactamase positive group (p=0.211). The results of the treatments for the different treatment groups are shown in Table 1. The number of post-treatment samples (1 or 2) had some effect on the results: In 4 out of 38 cases with no cure growth of *S. aureus* was detected 2 weeks after treatment but not 4 weeks after treatment. On the other hand, in 11 cases *S. aureus* growth was detected in the second but not in the first follow-up sample. All quarters with bacteriological cure were also clinically cured. The results for clinical cure are not given separately.

The bacteriological cure rate was significantly higher in the cows in their first lactation compared with subsequent lactations (p = 0.027) in the *β*-lactamase negative group, but not in the *β*-lactamase positive group (p=0.886). In the

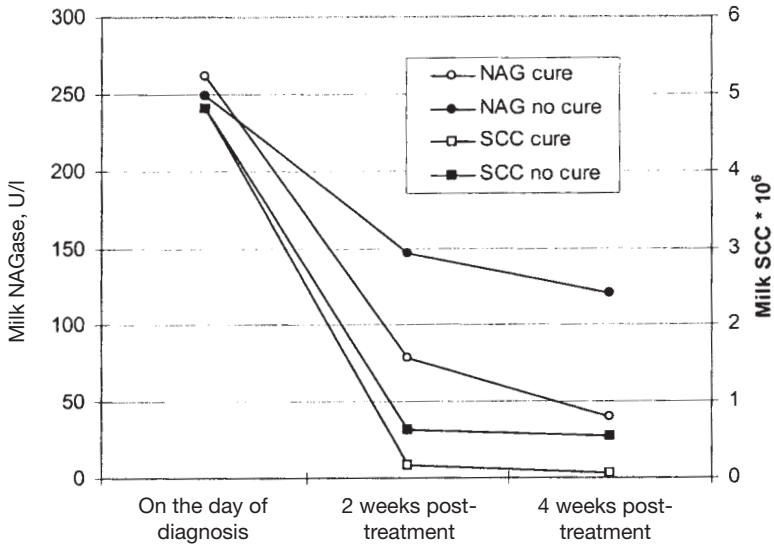

Figure 1. Mean milk SCC and NAGase activity in the quarters with bacteriological cure vs no cure on the day of diagnosis and at 2 and 4 weeks post-treatment. On the day of diagnosis, milk SCC was estimated using the California Mastitis Test.

first lactation cows with mastitis caused by *ß*-lactamase negative bacteria, 22 out of 24 quarters (91.7%) and in older cows, 69 out of 103 quarters (67.0%) were bacteriologically cured. In the group with mastitis due to penicillin-resistant strains, 3 out of 7 cases (42.9%) of cows on their first lactation and 15 out of 32 cases (46.9%) of cows in subsequent lactations were cured. The stage of lactation had no effect on the cure rates, but the proportion of moderate or severe signs was significantly (p=0.003) higher (80.3% vs. 58.1%) during the first 14 days post-partum than later in lactation. The severity of the clinical symptoms on the day of diagnosis did not affect the cure rate, although the proportion of cows with moderate or severe signs was slightly higher in the group that did not recover bacteriologically (71.4% vs. 65.7%).

When an indicator of inflammation (milk NAGase activity) was included in the criteria of cure, cure rates were lower. The total cure rate (bacteriological, clinical + milk NAGase <40 U 4 weeks post-treatment) in the penicillin-susceptible group was 55.9% and in the penicillin-resistant group 17.9% (Table 1). This difference is highly significant ($p<0.001$). The treatments did not affect the total cure rates significantly in either *ß*-lactamase group. Of the clinically cured quarters, 28% were not cured bacteriologically, and in 38% of them milk NAGase activity was above the threshold level. Milk NAGase value and CMT score on the day of diagnosis did not affect the bacteriological cure rate. Inflammatory reactions in the udder, determined with milk NAGase activity and SCC score, decreased clearly during the follow-up period in the bacteriologically cured quarters but remained at higher levels in quarters with no bacteriological cure. Fig. 1 shows milk

NAGase and SCC values in the combination treatment groups.

Discussion

The most significant factor affecting the cure rates for clinical *S. aureus* mastitis was the ability of the isolate to produce *ß*-lactamase. This has also been shown by other authors (*Sol et al.* 2000), and could indicate either that penicillin-resistant strains are more virulent than penicillin-susceptible strains, or that the antibiotics used to treat mastitis caused by penicillin-resistant strains are less efficient, due to pharmacokinetic or pharmacodynamic factors. The latter explanation was suggested by *Ziv & Storper* (1985), who studied subclinical staphylococcal mastitis and also found inferior cure rates for mastitis due to penicillin-resistant isolates compared with cases caused by penicillin-susceptible isolates. In the study of *Sol et al.* (2000), clinical *S. aureus* mastitis caused by *ß*-lactamase positive or negative isolates was treated intramammarily with 5 different antimicrobial treatments. The isolates were found to be in vitro susceptible to the drug used. No difference in the bacteriological cure rates between the different antimicrobial treatments in either group was found, but the difference in bacteriological cure rates between mastitis due to *ß*-lactamase positive and negative strains was significant. *S. aureus* is known to possess many virulence factors, like capsule and slime formation, which make them more resistant to antimicrobial treatment (*Sandholm et al.* 1990, *Baselga et al.* 1994). The possible relationship between production of *ß*-lactamase and other virulence factors of *S. aureus* has, to our knowledge, not been investigated.

Systemic treatment was introduced to mastitis therapy decades ago on the basis of experimental studies (*Ullberg et al.* 1958, *Ziv* 1980). The superiority of systemic treatment over intramammary treatment has never been proven in comparative clinical trials. However, based on published information from separate studies, there is some evidence that systemic treatment may be more efficient in *S. aureus* mastitis (*Ziv & Storper* 1985, *Aungier & Austin* 1987, *Owens et al.* 1988, *Pyörälä & Pyörälä* 1998, *Deluyker et al.* 1999, *Knight et al.* 2000). Mastitis caused by *S. aureus* is often intracellular and penetrates deep into the tissue. Drugs that are administered systemically penetrate better into infection foci (Ziv 1980). As regards the pharmacokinetics of the antimicrobials used here, with parenterally administered penicillin G at the dosing we used, therapeutic concentrations in the udder for susceptible *S. aureus* strains can be maintained (*Franklin et al*. 1986). Spiramycin penetrates well into the udder and milk when administered parenterally at the dosage used in this study (*Franklin et al.* 1986), but milk strongly reduces its antibacterial activity (*Louhi et al.* 1992). Information about the pharmacokinetics of systemic amoxycillin-clavulanic acid suspension in dairy cows is almost totally lacking, but in view of the low dose used here as recommended by the manufacturer and according to the literature which is available (*Prescott et al.* 2000), therapeutic concentrations could hardly be achieved in the udder. The treatment effect found here was thus mainly based on the intramammary component. Intramammary treatment given to supplement systemic administration of antimicrobials increases drug concentration in the milk compartment, and higher concentrations throughout the mammary gland will follow (*Ullberg et al.* 1958, *Ziv* 1980). In theory, combination treatment thus should improve cure rates for deep infections such as *S. aureus* mastitis (*Sandholm et al.* 1990). On the other hand, *ß*-lactam antimicrobials are time-dependent drugs, and very high concentrations at the infection site do not increase efficacy (*Craig* 1993). Our results show that in cases of mastitis caused by *ß*-lactamase negative *S. aureus* strains, combined

treatment with penicillin G was more efficient than systemic treatment alone. Previous studies on the effect of combination treatments are very scarce. In one experimental study with limited material, *Owens et al.* (1988) found higher cure rates in *S. aureus* mastitis as compared with intramammary treatment only. No information about *ß*-lactamase production of the isolate was available in that study. In some extensive field trials carried out in Scandinavia, systemic or short-term combined treatment in mastitis due to penicillin G susceptible agents has been studied (*Funke* 1982, *Jarp et al.* 1989, *Waage* 1997, *Pyörälä & Pyörälä* 1998). As regards mastitis derived from penicillin-susceptible *S. aureus* strains, *Jarp et al.* (1989) found in Norway that a 59.5% bacteriological cure rate could be achieved using 5-day systemic treatment with procaine penicillin G, which is in agreement with our results. *Waage* (1997) studied 5-day intramammary treatment with penicillin G that was supplemented parenterally for 1 or 3 days, but did not find any statistical differences between the 2 regimens. The average bacteriological cure rate in that study was 52%, which is very close to our results with systemic treatment. The reason for the finding that no advantage was achieved with the combination treatment over the 3-day period remains unclear. Recently, the therapeutical effects of parenteral, intramammary and combination treatments with amoxycillin-clavulanic acid have been compared (*Perner et al.* 2002). The study material consisted of 376 mastitis quarter cases, 159 of them caused by *S. aureus* and 32 by coagulase-negative staphylococci. The ability of *ß*-lactamase production of the staphylococci was not tested. Combination treatment was found to be superior over parenteral and intramammary treatment only. For all causing agents and mastitis types (acute, subclinical and chronic), the bacteriological cure rate was 75.3%, whereas the clinical cure, which included CMT-test, was only 40.0%.

Different criteria used to assess cure make comparison of different treatment trials difficult (*Pyörälä* 1988). In this study, strict criteria were used to calculate total cure rates, which were clearly lower than the bacteriological cure rates. Inclusion of a marker indicating the inflammatory status of the quarter could be useful, at least if only one follow-up milk sample is taken for bacteriological examination. The follow-up sample should not be taken earlier than 4 weeks after the beginning of the treatment, because growth of *S. aureus* may be suppressed. Our results agree with other authors' findings (*Neave* 1975) in this respect. In our study, it was clearly shown that in quarters with bacteriological cure, milk NAGase activity decreased to the threshold level by 4 weeks post-treatment (Fig. 1).

Many manufacturers of mastitis preparations have focused on broad spectrum antibiotics that could be used to treat all mastitis cases, regardless of the causing agent or the antimicrobial susceptibility of the pathogen (*Prescott et al.* 2000). At the same time an effort is being made to limit the use of broad-spectrum antibiotics, since their extensive use might promote antibiotic resistance (*Anonymous* 1996, *Anonymous* 1998). The cure rates for mastitis caused by *S. aureus* using broad-spectrum antibiotics have in many studies been inferior to our cure rates with penicillin G (*Wilson et al.* 1996, *Owens et al.* 1999). Penicillin G can be recommended as the drug of choice in mastitis due to penicillin-susceptible *S. aureus*. At the time of this study, intramammaries containing penicillin G alone were not available. The therapeutic effect of intramammaries containing penicillin G and an aminoglycoside on *S. aureus* is, however, mainly based on the penicillin G component, and the therapeutic effect of aminoglycoside in the combination is negligible (*Ødegaard & Sviland* 2001, *Taponen et al.* 2003). It seems evi-

dent that the treatment regimens using antimicrobials currently available for mastitis during lactation are not effective against mastitis caused by penicillin-resistant *S. aureus*. As regards *S. aureus* mastitis in general, good milking hygiene, culling of infected cows and drying-off of the chronically infected quarters are more effective means to control spreading of infection and to decrease the incidence of mastitis in the herd (*Saperstein et al.* 1988). Testing for *ß*-lactamase production of staphylococci isolated from mastitis should be included in practice as a routine method since it brings valuable information concerning prognosis and prevention strategies of mastitis in the herd.

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Sammendrag

Effekten av en riktad 5 dagars kombinerad parenteral och intramammar behandling av klinisk mastit förorsakad av pencillinkänslig och penicillinresistent Staphylocuccus aureus.

En kombination av parenteral och intramammär behandling av mastit jämnfördes med endast parenteral behandling. Studien omfattade kor med klinisk mastit (166 juverdelar med mastit) orsakad av *Staphylococcus aureus.* Korna behandlades av veterinärer från veterinärmedicinska fakultetens ambulatoriska klinik under normala gårdsbesök. Behandlingen baserade sig på in vitro känslighetstestning av bakterieisolaten. Procaine penicillin G (86 fall orsakade av *ß*-laktamas-negativa isolat) eller amoxycillin-clavulan syra (24 fall orsakade av *ß*-laktamas-positiva isolat) administrerades parenteralt i 5 dagar och intramammärt i 4 dagar. Behandlingens effektivitet kontrollerades 2 och 4 veckor senare genom klinisk undersökning, bakteriologisk odling och undersökning av somatiskt celltal och NAGase-aktivitet i mjölken. Juverdelar med *S. aureus* -växt i det ena eller båda proverna tagna efter behandlingen klassificerades som icke-tillfrisknade. Som kontroller användes 41 fall av klinisk mastit orsakade av penicillinkänsliga *S. aureus* -isolat, som behandlats parenteralt med procaine penicillin G i 5 dagar och 15 fall orsakade av penicillinresistenta isolat, som behandlats parenteralt med spiramycin i 5 dagar, båda från samma praktikområde som testgruppen. Bakteriell avläkning efter kombinationsbehandlingen var 75.6% för juverdelar infekterade med penicillinkänsliga *S. aureus* -isolat och 29.2% för juverdelar infekterade med penicillinresistenta isolat. Tillfriskningsgraden för fall behandlade parenteralt med endast procaine penicillin G var 56.1% och för fall behandlade med spi-

ramycin 33.3%. Skillnaderna i bakteriell avläkning mellan mastit orsakad av penicillinkänslig och penicillinresistent *S. aureus* var starkt signifikant. Behandlingen inverkade signifikant på tillfriskningsgra-

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