Kafkas Univ Vet Fak Derg
16 (1): 97-104, 2010
DOI:10.9775/kvfd.2009.501

RESEARCH ARTICLE

The Efficacy of a Cefoperazone - Danofloxacin Combination in the Treatment of Subclinical Mastitis in Dairy Cows Caused by *Staphylococcus aureus* [1]

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[1] *This work was supported by the Adnan Menderes University, Scientific Research Projects Commission VTF-011009, Aydin - TURKEY*
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Makale Kodu (Article Code): KVFD-2009-501

Summary

We report on the effectiveness and profitability of a single-dose intramammary administration of cefoperazone and danofloxacin combination (CDC) against subclinical mastitis in lactating cows. The CDC proved to be effective against *Staphylococcus aureus (S. aureus), in vitro* checkerboard trials. The presence of mastitis examined on the 892 mammary quarters of 223 lactating cows by bacterial cultures and microscopic somatic cell counting (SCC). Selecting the subjects at random from the 223 animals, twenty-two cows (32 quarters) used for treatment and eleven cows (15 quarters) as controls. Milk samples received one week before the start of treatment and on days 0, 14, 21 and 28 of the experiment. Bacteriological cure examined to the treated quarters. Bacteriological cure defined as no signs of *S. aureus* after treatment of quarters previously confirmed as infected. The SCC remained unchanged 4 weeks after treatment started, but quarters that were cured (spontaneously or by CDC) showed significant differences on days 21 and 28 ($P<0.05$). There was no *S. aureus* in 53.1% of the treated quarters with intramammary CDC, while in the controls there was spontaneous cure of the infection in 13.3% of the untreated quarters with significant ($P<0.05$) difference. These preliminary results suggest the intramammary application of CDC is an effective and profitable therapy in treating *S. aureus* infection resulting in subclinical mastitis in lactating cows.

Keywords: *Subclinical mastitis, Cefoperazone, Danofloxacin, Combination, Treatment, Staphylococcus aureus*

Sütçü İneklerde *S. aureus*'un Neden Olduğu Subklinik Mastitisin Sağaltımında Sefoperazon - Danofloksasin Kombinasyonunun Etkinliği

Özet

Sütçü ineklerde *S. aureus*'un neden olduğu subklinik mastitiste meme içi tek doz uygulanan sefoperazon ve danofloksasin kombinasyonunun (CDC) etkinliği bu çalışmada araştırıldı. CDC etkinliği in vitro olarak *S. aureus*'a karşı checkerboard testiyle kanıtlandı. Mastitisin varlığı 223 sağım dönemindeki ineğin 892 meme bölümünde bakteri kültürü ve doğrudan mikroskopik somatik hücre sayımı (SCC) ile belirlendi, rastgele seçilen 22 inek (32 meme bölümü) sağaltım grubu ve 11 inek (15 meme bölümü) kontrol grubu olarak ayrıldı. Denemeden 1 hafta önce ve sağaltımın 0, 14, 21 ve 28. günlerinde süt örnekleri alındı. Sağaltıma alınana meme bölümlerinde bakteriyolojik iyileşme incelendi. Bakteriyolojik iyileşme daha önce saptanan *S. aureus*'un sağaltım sonrası saptanmaması olarak kabul edildi. Sağaltım grubunda SCC sağaltımın 4. haftasında değişmeden kaldı, iyileşen meme bölümlerinde (kendiliğinden veya CDC uygulanan) denemenin 21. ve 28. günlerinde anlamlı farklılık vardı ($P<0.05$). Meme içi CDC ile sağaltıma alınan meme bölümlerinden %53.1'de *S. aureus* saptanmadı, ancak; kontrol grubu meme bölümlerinde %13.3 oranında kendiliğinden iyileşme saptandı ve bu fark anlamlıydı ($P<0.05$). Elde edilen bu sonuçlar sağılan ineklerde meme içi CDC uygulamasının *S. aureus*'un neden olduğu subklinik mastitislerde etkili olabileceğini gösterdi.

Anahtar sözcükler: *Subklinik mastitis, Sefoperazon, Danofloksasin, Kombinasyon, Sağaltım, Staphylococcus aureus*

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INTRODUCTION

Mastitis is an endemic disease considered to be one of the most frequent and costly diseases in the dairy industry. Contagious mastitis often involves infections by *Staphylococcus aureus (S. aureus)* [1-4]. It is the most common pathogen in dairy herds and contributes to milk loss, reduced milk quality and chronic infections throughout the world [5-7]. The virulence varies with different strains of the bacteria and herd susceptibility [8-10]. The cure rates for *S. aureus* mastitis vary; for example, cure rates for subclinical *S. aureus* mastitis range from 3.6% to 92.0%, depending on the race of the cattle, bacterial strain and method of treatment [11-13].

There is increased awareness about the excessive use of antibiotics in farming, especially animals intended for human consumption. One such case is treating clinical mastitis in dairy cows [11,14]. Intramammary treatment with combinations of two or even three antibiotics is part of mastitis therapy to cover a wider range of pathogens, decrease emerging resistant strains and possibly increase the activeness of the antimicrobials because of synergistic or additive effects. It is also showed that a combination of antibiotics reduces toxicity by requiring lower doses of individual parts [9,15,16].

Subclinical *S. aureus* mastitis is rarely treated during lactation because it is widely believed to be uneconomical, although there are no economic studies that support this view [4,17]. When treatment of *S. aureus* mastitis is postponed until the dry period that may origin duration of infection increases, shedding bacteria and somatic cells, palpable tissue changes, and infection of multiple quarters within a cow. However, mastitis has been associated with an increased risk of culling [2,18]. Some authors [17,19] state that better cure rates gained by treating subclinical infections in lactation than by treating clinical cases of *S. aureus* mastitis.

The literature is not uniform about treatment regimes. The results depend on the used antibiotic, susceptibility of the organisms, the length and the way of treatment, somatic cell count (SCC) method, the age, the number of quarters infected and immune status of the cow, the duration of the subclinical *S. aureus* infection, the time between treatments and the final sampling of the treated quarter [10,12,19]. Extended therapy is significantly more effective at eliminating natural and experimentally induced mammary duct infections than standard intramammary treatments [10,11,20-22]. The benefits of extended therapy protocols, such as improved marketability of milk, better rates of bacteriological cure and lower SCC and of risks of transmission must be weighed against its drawbacks. Which include higher costs of the antibiotics, loss of milk due to withdrawal, increased risk of antibiotic residue in the milk, and the potential of infecting the cow through repeated infusions by the teat canal [11,20].

The economic impact of bovine mastitis and intra-mammary infections led to developing various therapeutic strategies to control intramammary infections. Many drugs belonging to various therapeutic classes, anti-microbials, antiinflammatory drugs, vitamins, vaccines, cytokines and even homoeopathy have been assessed as well as several routes of administration [5,23]. Combination of anti-microbial agents for treating infections due to *S. aureus* used to increase the antibacterial activity and to prevent the emergence of drug resistance [24]. The cefoperazone and danofloxacin antibacterials were selected at the present study by their pharmacokinetic and pharmacodynamic characteristics [25-27]. Combination of fluoroquinolones with other antibacterial agents have been extensively researched [16,28-31]. Combinations of fluoroquinolones with aminoglycosides, β-lactams, imidazoles, macrolides and clindamycin infrequently show synergy against *Enterobacteriaceae spp.* and gram-positive bacteria, the combinations rarely show antagonism [25]. Cefoperazone is a third-generation cephalosporin that inhibits bacterial cell wall formation. It is resistant to the effect of β-lactamases produced by certain pathogens of the Staphylococci family [27,32]. Danofloxacin is a fluoroquinolone developed specifically for the use in domesticated animals. It acts by inhibition of bacterial DNA-gyrase. Both cefoperazone and danofloxacin are effective against gram-negative and gram-positive bacteria [16,25,26,33].

Economical considerations require the best possible cure should obtain with the shortest possible withdrawal period as a result the milk can be marketed again as soon as possible [2,18,22]. In this case, the antibiotic must have a low degree of binding to milk and udder tissue proteins to ensure a fast rate of drug distribution to the various parts of the udder. Also, the vehicle must ensure fast and even distribution of the antimicrobial drug [23]. Dimethylsulphoxide (DMSO) was chosen as vehicle of antibiotics in this study. DMSO is one of the earliest and most widely studied penetration enhancers [34]. Aprotic solvents such as DMSO, dimethylformamide and dimethyl-acetamide accelerate the permeation of many drugs including antifungal agents, antibiotics, barbiturates, steroids, and local anaesthetics [35].

The objective of this study was to evaluate effectiveness and profitability of a cefoperazone and danofloxacin combination (CDC) antibiotic agents against subclinical *S. aureus* mastitis in milk cows, with emphasis in minimizing antibiotic use.

MATERIAL and METHODS

Cows and Study Design

The study conducted according to the European Commission guidelines on Good Clinical Practices for the conduct of clinical trials for veterinary medicinal products, Guideline for the Conduct of Efficacy Studies for Intramammary Products for Use in Cattle and with strict adherence to the guidelines of the Adnan Menderes University for experiments involving animals under veterinary care (Decision date 17.11.2003 and number HEK/2003/0032).

The animals were part of a herd of 223 lactating Holstein cows from a farm located within 35 km of the Faculty of Veterinary Medicine, Adnan Menderes University in Aydin, in South-western Turkey. All the cows fed with antibiotic free grain mixture concentrate, corn silage and clover hay. They milked twice daily in a computer-controlled tandem-milking parlour provided with a recorder from DeLaval, Sweden. The mean age of the cows was 5 years; the median day in milking was 157 days. Except for the treatment with antibiotics, both the study subjects and controls maintained in identical conditions.

All lactating cows with presence of *S. aureus* with quarter CMT ≥2 and Log-transformed direct microscopic SCC ≥14.0 prior 1 week beginning of the experiment were included to the study. Cows with visible teat damage, signs of disease, clinical mastitis as well as intercurrent diseases and received any drug treatment within a 30-day period before the experiment excluded from the experiment. Entitled cows randomly separated into a study group (n = 22) and a control group (n = 11).

Milk Sampling

Milk samples aseptically collected from cows quarters seven days before treatment (-7). Milk samples also collected at the moment of application of the antibiotics day 0 and at days 14, 21 and 28 after the treatment. Before sampling, teats cleaned by dipping in an iodine-based germicide and the teat orifice scrubbed thoroughly using cotton wool soaked in 70% ethyl alcohol and the first three streams of milk discarded. All samples were aseptically collected before milking from each quarter of cows enrolled in the study. Additional quarter milk samples collected for SCC. All milk samples were refrigerated until needed for analysis.

Laboratory Procedures

Cow's milk samples of the each quarter in the herd screened by the California Mastitis Test (CMT) and direct microscopic SCC measured with a Leica QWin software (Leica Microsystems, Wetzlar, Germany) with the methods described by Schalm et al.[36]

Isolation and identification of bacteria the milk inoculated on agar plates containing 5% defibrinated ovine blood (Oxoid Limited, Hampshire, United Kingdom) and incubated at 37°C for 24-48 h. The suspected colonies were gram-stained and examined under the microscope. Gram-positive cocci further identified by biochemical tests. Identification of *S. aureus* carried out by colony morphology, Gram staining and biochemical tests. Biochemical characteristics of the isolates determined by using catalase, haemolysis on blood agar, coagulase, nitrate reduction, DNAse agar, clumping factor, arginine dihydrolase, urease and novobiocin resistance tests. In addition, fermentation tests, such as production of acid from sucrose, maltose, D-mannitol, D-trehalose, and raffinose also carried out [37,38].

The antimicrobial susceptibility tests for *S. aureus* performed on Mueller-Hinton agar plates (n = 47) following the disc diffusion susceptibility test procedures as recommended by the National Committee for Clinical Laboratory Standards [39] and the disc manufacturer's instructions (Difco Laboratories, Detroit, USA; Oxoid Limited, Thermo Fisher Inc, USA).

Cefoperazone and Danofloxacin Dombination

Standard antibiotic powders, danofloxacin mesylate (lot No: 53665342) and cefoperazone sodium (lot No: 606-356011) provided by Pfizer Ltd Sti, Istanbul, Turkey.

The *in vitro* interactions between the two selected antibiotics studied in checkerboard titration experiments [15]. Checkerboard titration experiments performed in triplicate in 96-well U type microtiter trays. The degree of interaction between cefoperazone and danofloxacin was determined by calculating the fractional inhibitory concentrations index (FIC index). FIC index calculated as follows:

FIC index = FIC of cefoperazone + FIC of danofloxacin

The FIC of cefoperazone and danofloxacin was calculated as follows:

FIC of cefoperazone = MIC of cefoperazone in combination /MIC of cefoperazone alone

FIC of danofloxacin = MIC of danofloxacin in combination /MIC of danofloxacin alone

Synergy defined as an FIC index ≤0.5, additivity or indifference defined as an FIC index >0.5 and antagonism defined as an FIC index >4 [15]

The FIC index for *S. aureus ATTC 25923* was 0.25[40]

Following the *in vitro* results, an antibacterial combination was prepared containing 37.5 mg danofloxacin and 75.0 mg cefoperazone[40] per 10 ml in a 50% DMSO-water mixture (v/v). The quarters of the cows to be treated infused once immediately after milking with the antibiotic combination while the controls received 10 ml saline solution. The cows not milked for at least 8 h after infusion.

Definition of Cure

The outcome of the experiments assessed four weeks after treatment. On the follow-up visits the cows clinically evaluated and milk samples were taken from the quarters. Mammary glands without clinical abnormalities yielding apparently normal milk that were CMT (≥2), direct microscopic SCC (LnSCC ≥14.0) and bacteriologically positive were diagnosed with subclinical mastitis. The bacteriologic cure of the quarter defined as the absence of *S. aureus* colonies on days 14, 21 and 28 after the treatment started.

Statistical Analysis

The chi-square Fisher's exact test used to find out bacteriological cure differences between groups. A two-sample t-test was used to evaluate initial LnSCC of the two groups. The paired t-test used for evaluation of differences between repeated measures of LnSCC previous to onset of subclinical mastitis. The statistical significance set at P<0.05. All data checked for missing and unlikely values; errors were checked. Quarters without inclusion criterion or with incomplete data excluded from statistical analysis during the study period.

RESULTS

Eight hundred ninety quarters of 223 cows evaluated by the CMT. At the beginning of the experiment, 26% of the examined quarters (232/892) were mastitis. Of these, 31.5% (73/232) quarters were infected with *S. aureus.* Age and lactation day of cows were 4.93, 5.16 and 152, 162 for control and treatment groups, respectively. Direct microscopic mean LnSCC quarters of treated and control groups were 14.03±0.33 and 14.06±0.18, respectively.

The results of the antimicrobial susceptibility tests were shown in *Table 1*. Most of *S. aureus* strains were susceptible to both cefoperazone and danofloxacin. The results of CDC therapy presented in *Table 2*. Overall, CDC therapy resulted in bacteriologic cure in 53.1% (n = 17) quarters, compared with only 13.3% (n = 2) in the untreated controls (P<0.05).

Table 1. *Per-Cent susceptibilities of S. aureus strains (Both CDC treated group and control group, n=47) isolated from cows with subclinical mastitis to various antibiotics*

Tablo 1. *Subklinik mastitisli ineklerden izole edilen S. aureus suşlarının (CDC sağaltım grubu ile kontrol grubu beraber, n=47) çeşitli antibiyotiklere olan % duyarlılıkları*

Susceptible	Resistant	Intermediate	Susceptible
Cefoperazone (Oxoid, 75 µg)	6.4	10.7	82.9
Danofloxacin (Difco, 5 µg)	6.4	0	93.6
Nalidixic acid (Oxoid, 30 µg)	12.8	42.6	44.6
Penicillin G (Oxoid, 10 IU)	17.0	23.4	59.6
Amoxicillin (Difco, 20 µg) + Clavulanic acid (Difco, 10 µg)	0	6.4	93.6
Cefuroxime (Oxoid, 30 µg)	0	8.5	91.5
Neomycin (Oxoid, 10 µg)	34.1	51.1	14.8
Erytromycin (Oxoid, 15 µg)	0	44.7	55.3
Tetracycline (Oxoid, 30 µg)	0	23.4	76.6
Lyncomycin (Oxoid, 15 µg)	14.9	72.4	12.7

Table 2. *Intramammary application of cefoperazone and danofloxacin combination: bacteriological cure rates of subclinical S. aureus mastitis*

Tablo 2. *Meme içi uygulanan sefoperazon ve danofloksasin kombinasyonu: Subklinik S. aureus mastitiste bakteriyolojik iyileşme oranları*

CDC Treated Group		Controls	
Number of Treated Quarters	Cured Quarters (%)	Number of Quarters	Spontaneous Cure (%)
32	17* (53.1)	15	2 (13.3)

** P<0.05*

The LnSCC values were presented in *Table 3*. The first values were similar in both groups. However, LnSCC remained unchanged 4 weeks after treatment started, quarters that were cured (spontaneously or by CDC) showed significant differences on days 21 and 28 (P<0.05).

Table 3. *Ln somatic cell counts: Cefoperazone and danofloxacin combination treated and untreated quarters and in cured and non-cured quarters*

Tablo 3. *Ln Somatik hücre sayıları: Sefoperazon ve danofloksasin kombinasyonu ile sağaltılan ve sağaltılmayan meme bölümleri ile iyileşen ve iyileşmeyen meme bölümleri*

Days	Untreated Quarters (n= 15)	CDC Treated Quarters (n= 32)	P	Non Cured Quarters (n= 28)	Cured Quarters (n= 19)	P
0	14.03±0.33	14.06±0.18	0.701	14.25±0.19	13.95±0.28	0.366
14	14.34±0.29	14.34±0.19	0.992	14.55±0.18	14.04±0.27	0.106
21	14.54±0.35	14.04±0.20	0.196	14.61±0.22	13.65±0.26	0.007*
28	14.37±0.40	14.00±0.20	0.368	14.58±0.23	13.50±0.26	0.003*

** P<0.05*

DISCUSSION

To our knowledge, this is the first report describing the use of a combination of cefoperazone and danofloxacin

for the treatment of *S. aureus* subclinical mastitis as well as other pathogens in dairy cows.

Antibacterial susceptibility testing used as a basis for therapy selection [5,8,11]. In the early days of mastitis therapy penicillin was widely used but soon many strains of *Staphylococcus spp.* became resistant to penicillin as they produced and secreted penicillin-destroying β-lactamases [5,14]. Cefoperazone is resistant to the effect of β-lactamases produced by certain *Staphylococci spp.* and danofloxacin has no β-lactam ring [25,27]. Most of isolated *S. aureus* strains were sensitive to cefoperazone and danofloxacin antibiotic agents *(Table 1),* as well as cefoperazone and danofloxacin (or fluoroquinolones) were found to be effective against *S. aureus* mastitis [8,41,42], which is consistent with our results. Similarly to Owens et al. [42] study, as a result of better bacteriologic cure rate with antibiotics, *S. aureus* isolates were sensitive to cefoperazone and danofloxacin. Selection of antibiotics for treatment based *in vitro* susceptibility to antibiotics is no guarantee for treatment success *in vivo* and *in vitro* testing. Susceptibility results can be used as a predictor for cure for *S. aureus* infections of less than 2 week duration, but not for chronic intramammary infections [5,11]. The duration of infections at the present study was approximately 1 week in duration. Data of the present study support agreement between results of antimicrobial susceptibility tests and clinical efficacy in short term *S. aureus* intramammary infections as other researches [12,24,42].

Little research has focused on treatment of cows with subclinical mastitis during lactation [21]. Because, treatment of subclinical *S. aureus* mastitis during lactation is generally considered ineffective and economically not justified [17]. Although, cows subclinically infected with *S. aureus* are a permanent source of infection for other dairy cows, this infection may potentially increase the incidence of intramammary infections. Shortening the duration of intramammary infections by culling and antimicrobial therapy are important components of a programme for mastitis management [12]. Comparison of our results for bacteriologic cure of subclinical *S. aureus* mastitis during lactation to other limited studies is difficult because of differences in bacteria, antibiotics and study designs. Although the sample sizes of groups in the present study are relatively small, our results indicate that single dose intramammary CDC therapy was more effective 53.1% (17/32) in eliminating *S. aureus* infections. While, our spontaneous remission cases in the untreated control group 13.3% (2/15) was similar to Oliver's results [21].

An increase in the dose and duration of lactational antibiotic therapy has been shown to improve cure rates as has the combination of intramammary and intramuscular routes of therapy [13,21,43]. However, these tactics often fail to provide sufficiently high cure rates during lactation [44]. For example, Sol et al.[7] studied 3 antibiotic combinations (ampicillin - cloxacillin, cephalotin - colistin and rifamycin - trimethoprim) as well as oxyminopenicillin and cefazolen. They [7] obtained cure rates for clinical *S. aureus* mastitis 50% (42/84), 57% (17/30), 56% (10/18), 46% (25/54) and 67% (10/15), respectively. In all cases three intramammary infusions applied at 12-h intervals. If the results were not considered to be satisfactory, the herd keepers were asked to continue the treatment for an additional 48 h, also at 12-h intervals. Repeated antibiotic infusions with extended therapy protocol proved to have no additional benefits [7]. In contrast, the differences in clinical stage of trials we tried only a single intramammary CDC infusion to the present study.

S. aureus cure related to the duration of infection, number of quarters infected, drug chosen, length of time the drug administered, and immune status of the animal [5,10,11]. Owens et al.[44] administered tilmicosin (intramammarily 300 mg x 6 consecutive milking or 3.000 mg subcutaneously once per day 3 times) and ceftiofur (intramammarily 200 mg x 6 consecutive milking or 500 mg intramuscularly once per day 3 times) against *S. aureus* mastitis in lactating cows. Results showed that only one quarter (1/11) was cured by intramammary infusion of tilmicosin at 28-day post-treatment. However, researchers cited that tilmicosin was effective when used as a dry cow product against *S. aureus,* the cure rate was 74%. Although, the cure rates for lactation therapy are lower than for dry-cow treatment [12,14,43,45]. We did not evaluate the CDC for dry-cow therapy, but lactational cure rates were superior to Owens et al.[44] study with single intramammary infusion. Other study conducted by Oliver et al.[21] evaluating the efficacy of ceftiofur for treatment of subclinical mastitis in lactating dairy cows resulted with a cure rate for *S. aureus* was 7% for 2-day, 17% for 5-day and 36% in the 8-day extended therapy. They [21] reported the best cure rate was 36% (4/11) for *S. aureus* on the 8th day of therapy and the spontaneous cure rate was 11% (4/38). Present study results were more beneficial compared with Oliver et al.[21] study and spontaneous cure rate was similar.

Owens et al.[42] studied the antimicrobial susceptibility of penicillin, novobiocin, the penicillin and novobiocin combination, and the other antibiotics as well comparison of therapy success for bovine mastitis bacteria during lactation. Infected quarters treated with a commercial combination consisted of 100.000 U of procaine penicillin and 150 mg of novobiocin infused two times intramammarily at 24-h intervals. Bacteriologic cure rates for newly acquired *S. aureus* intramammary

infection (<2 week in duration) at 28-day post-treatment were 70% and cure rates for chronic *S. aureus* intramammary (>4 week duration) were 35%. But, effects of spontaneous cures on therapy results were not determined. Present study *S. aureus* intramammary infections were newly acquired (approximately 1 week in duration) infections and bacteriologic cure rate was 53.1% at 28-day post-treatment with a single intramammary infusion of CDC. Duration of *S. aureus* intramammary infections was approximately 1 week shorter than Owens et al.[42] study and bacteriologic cure rate was lower with a single intramammary CDC infusion.

Wilson et al.[22] evaluated 9007 cases of subclinical mastitis in cows and found that none of the antibiotic treatments differed significantly from untreated cure rate 49% (90/184) versus 43% (472/1088) for *S. aureus* mastitis infections. Bacteriologic cure rates for S. *aureus* mastitis treated intramammarily with amoxicillin, cephapirin, cloxacillin, erythromycin, hetacillin and penicillin antibiotics were 43.0%, 43.0%, 47.0%, 65.0%, 20.0% and 65.0%, respectively. Surprisingly bacteriologic cure rate of untreated *S. aureus* infected quarters was 43.0% which is higher than the present study found 13.1%.

SCCs are directly related to the inflammatory process of the mammary gland in individual cows and can be used to establish the general health status of the herd and its consequences on the quality of the raw milk [46]. The time required for SCC to decrease substantially after successful therapeutic intervention depends on the type of microorganism involved and the tissue damage from the infection [6,36]. Pyorola and Pyorola [6] reported that SCCs were acceptable to predict bacteriological cure was greatest in *S. aureus* infection cases. But, SCCs were less specific for streptococci and coliform bacteria because the counts remained high even after bacteriological cure had been attained [6]. The cure rate is higher for subclinically infected *S. aureus* quarters with a low SCC[13]. However, once intramammary infection is diagnosed, the use of non-lactating as well as lactating cow therapy has proven highly effective in curing disease and in many herds, successful therapy reduces SCC and increases milk yield [47]. Successful treatment does not necessarily lead to a reduction in SCC[47]. The CDC did not reduce the quarter LnSCC in the treated group *(Table 3)*, but significant differences seen at quarters cured on the 21st and 28th days of the study. This decrease is consistent with those reported in other studies [4,19,48-50].

Preliminary results suggest that single dose intramammary application of a CDC appears to be an effective and profitable therapy in cases of subclinical mastitis caused by *S. aureus* in lactating cows. Present study involved only one herd with intramammary *S. aureus* infections that were newly acquired (approximately 1 week in duration) and one set of treatment protocols. Results cannot necessarily generalize to other herds using other treatments. More research involving in various herds is indispensable to find out the most efficient and cost-effective methods of treating subclinical mastitis caused by *S. aureus* in lactating cows.

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