Systematic Review: What is the Best Antibiotic Treatment for *Staphylococcus aureus* Intramammary Infection of Lactating Cows in North America?

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KEYWORDS

• Systematic review • *Staphylococcus aureus* • Bovine
• Mastitis • Treatment • Antibiotic • Lactation

Staphylococcus aureus is still one of the most prevalent pathogens causing intramammary infections (IMI) in dairy cattle worldwide.[1–6] *S aureus* is typically recognized as a cause of chronic subclinical infections with elevation of somatic cell count (SCC) but may also cause clinical mastitis.[7] In fact, it is the most prevalent pathogen found in clinical mastitis cases in Canada.[8]

Classic short-duration antibiotic therapy against *S aureus* IMI is often unrewarding because of low cure rates during lactation.[7,9–13] New treatment regimens, such as extended therapy or combination of local and systemic antibiotic therapy, have been studied in the past decade to try to improve those cure rates.[7,11,14–19] Cure rate improvements observed in some of these studies have stimulated the interest of producers and veterinarians in those treatment regimens.

However, the studies were performed in many different countries, on different breeds, with different management practices and using several different antibiotics and dosage protocols against subclinical or clinical *S aureus* IMI. Consequently, comparison between studies should be made with caution.

The authors have nothing to disclose.

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Evidence-based medicine aims to apply the best available evidence gained from the scientific method to clinical decision making. Systematic reviews aim to identify, evaluate, and summarize the findings of all relevant individual studies, thereby making the available evidence more accessible to decision makers.[20] There is no systematic review available to help veterinarians choose the best lactational antibiotic therapy against *S aureus* IMI.

The objective of this report was therefore to perform a systematic review to answer the following clinical question: What is the best antibiotic treatment for *S aureus* IMI of lactating dairy cows in North America?

MATERIAL AND METHODS

Search Strategy

To answer the question, the following keywords were used by the authors: *Staph aureus* or *Staphylococcus aureus*; antibiotic(s) or antimicrobial(s); treatment or therapy; mastitis or intramammary infection or intra-mammary infection; bovine or cow(s) or cattle or milking cow(s) or dairy cow(s) or heifer(s). The equation used in all databases was: [(bovine or cow(s) or cattle or milking cow(s) or dairy cow(s) or heifer(s)) and (mastitis or intramammary infection or intra-mammary infection) and (*Staph aureus* or *Staphylococcus aureus*) and (antibiotic(s) or antimicrobial(s))] or [(bovine or cow(s) or cattle or milking cow(s) or dairy cow(s) or heifer(s)) and (mastitis or intramammary infection or intra-mammary infection) and (*Staph aureus* or *Staphylococcus aureus*) and (treatment or therapy)].

The initial web search was conducted by only one of the authors (JPR) in CAB database (1973–2011 week 32), PubMed database, and MEDLINE database (1948–2011 August week 1). No restriction was applied. Those databases were accessed August 17, 2011.

Identification of Relevant Studies

For each database, all titles were examined by one author (JPR). If the title was clearly unrelated to the clinical question, the article was excluded for further exploration. Then, for all titles that could be related to the question, abstracts were read by the same person. Reasons for exclusions at that step were as follows: study unrelated to the clinical question (including antibiotics not available in North America), abstract not available, article not written in English, and article not published in a peer-reviewed journal. Finally, the remaining articles were read by both authors. Some articles were still excluded at this last step because they did not involve the use of antibiotics available in North America or because there were no data from an original research project included in the articles. Those late exclusions were necessary because it was not possible to do it earlier in the process since some important data were not mentioned in the title or the abstract. Flow charts were used to report the selection and exclusion process.

Antibiotics available in North America to the authors knowledge are (1) lactating intramammary antibiotics: pirlimycin hydrochloride, cephapirin sodium, cloxacillin sodium, amoxicillin trihydrate, hetacillin potassium, penicillin G procaine, ceftiofur hydrochloride, and erythromycin; (2) systemic lactating cow antibiotics: oxytetracycline hydrochloride, penicillin G sodium, penicillin G procaine, ceftiofur hydrochloride or sodium, erythromycin, trimethoprim-sulfadoxine combination, sulfadimethoxine, and ampicillin trihydrate; and (3) nonlactating cow antibiotics: florfenicol, tulathromycin, tylosin, tilmicosin, and enrofloxacin. Not all systemic lactating cow antibiotics are labeled to treat mastitis but as labels varies among countries, readers should validate this issue with their country regulators. Another product, only available in Canada, is

a combination of penicillin procaine, dihydrostreptomycin sulfate, novobiocin sodium, polymyxin B sulfate, hydrocortisone acetate, and hydrocortisone sodium succinate. No study was found using that specific formulation.

Selected Studies Assessment and Scoring

The remaining articles were assessed independently by both authors using the 100-point scaled version of the CONSORT 2010 checklist of information to include when reporting a randomized clinical trial (see article by Vandeweerd and colleagues elsewhere in this issue for further exploration of this topic). No attempt was made to obtain more details on any study by contacting researchers. A minimum score of 50 was used as the cut-off for inclusion. For articles with discordant results, the authors conducted a meeting to determine where scoring patterns diverged and to obtain a consensus.

Useful data on each article were recorded: authors, journal, year of publication, and country where the trial was performed. Data extracted on the methodology included randomization, blinding, treatment regimen, population studied, and group size. Outcome parameters studied were bacteriologic cure rates, new IMI rates, and post-treatment clinical mastitis incidence. Potential conflict of interest such as pharmaceutical company involvement as a funding source for the study or employer of at least one of the author was also recorded.

RESULTS

Figs. 1–3 shows flow charts of the study selection process. Briefly, 3082 titles were screened from the 3 databases. A total of 2889 titles were clearly unrelated to the clinical question and were excluded for further exploration. For the 193 titles that could be related to the question, 133 were excluded because of lack of relevance after abstract revision or because they were duplicates between database. An additional 40 articles were excluded because they were not published in English. Finally, the remaining articles (n = 20) were read by both authors.

Seven articles were excluded by the reviewers at this stage because they did not use antibiotics available in North America (n = 5) or because there were no data from an original research project included in the article (n = 2).

Of the remaining 13 articles, 5 articles were scored above 50 points by both reviewers for their quality and 5 articles were scored below 50 points. For the 3 articles with discordant results, consensus was reached after a discussion between the reviewers. As a result, 1 of the 3 articles was reclassified as meeting the criteria and the other 2 remained below the threshold. In total, 6 articles were included in the review.[9,14–16,19,21]

Table 1 summarizes the 6 articles that were retained. Five articles were randomized clinical trials with either a positive (treated) or negative (untreated) control. The sixth study was a large retrospective study, where cows were treated based on producer and veterinary treatment preference. In the retrospective study, treatment results were described for 7 antibiotics; however, only 4 antibiotics were used on more than 20 *S aureus* cases. Data for these antibiotic treatments are presented. Herds in all studies were commercial dairy herds with the exception of the study of Oliver and colleagues,[16] which used 3 University research herds. For Jarp and colleagues,[19] herd numbers were not specified, however, cases were recruited by 38 veterinary practitioners across 15 districts in Norway. For the retrospective study of Wilson and colleagues,[21] herd numbers were not specified; however, a very large number of screened samples from routine herd monitoring or high SCC investigations were included.

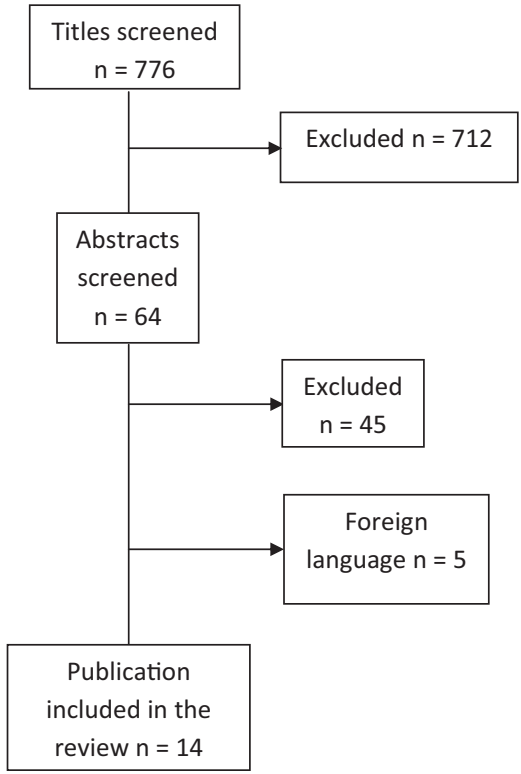

Fig. 1. Flow chart of selection process using PubMed database.

Recruitment practices and definitions of *S aureus* infection status at entry into the trial and definitions for cure of infection were different for each study. Some studies used milk culture–based diagnosis alone, while others used a combination of culture and SCC. The basic definitions are presented in **Table 2**. Four studies used subclinically infected cows only, 1 study used both subclinical and clinical cases, and 1 study used only clinically affected cows.

Definition of "infection cure" varied among the studies. Roy and colleagues[14] used the most restrictive definition for cure, requiring 3 negative follow-up cultures. Wilson and colleagues[9] required 3 negative cultures or 2 negative cultures and a low SCC. Oliver and colleagues[16] and Deluyker and colleagues[15] required 2 negative cultures to confirm cure, while Jarp and colleagues[19] and Wilson and colleagues[21] had only a single follow-up culture.

Results of treatment efficacy are presented in **Table 3**. Roy and colleagues[14] defined "cure" at both the quarter and cow level; however, full follow-up data were available only at the cow level because when a single quarter was found to be infected within the udder, sampling was discontinued for the entire cow. Conversely, for other studies cow level data were not presented. In the study of Roy and colleagues,[14] cow cure rate was higher (36.8%) for cows with one quarter infected versus 8.3% for cows with more than one quarter infected.

In general, cure rates for *S aureus* were low (<50%), with the exception of Deluyker and colleagues[15] and Wilson and colleagues.[22] Four of 6 studies showed significant

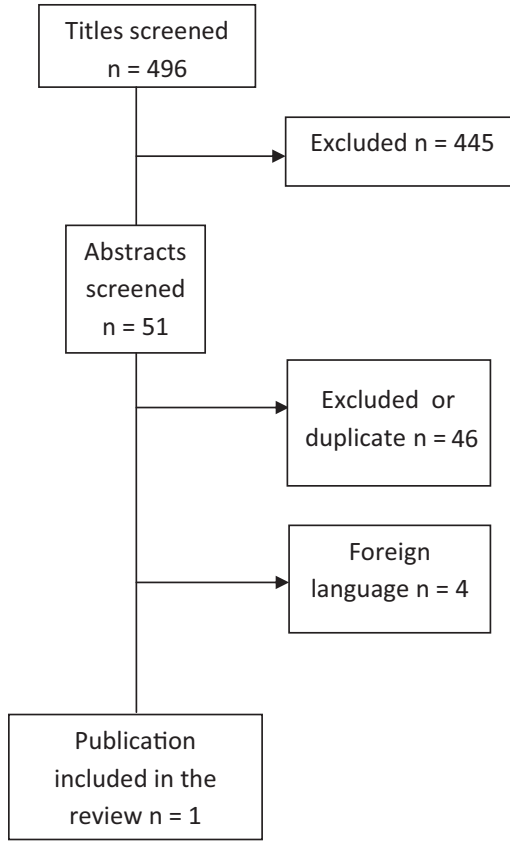

Fig. 2. Flow chart of selection process using MEDLINE database (1948–2011 August week 1).

treatment effects. Where duration of therapy with the same product was assessed (Deluyker and colleagues[15] and Oliver and colleagues[16]), longer-duration therapy had a positive association with cure rate. Only Jarp and colleagues[19] examined the impact of systemic versus systemic and intramammary therapy and, in fact, the product used for systemic therapy was different than the systemic/intramammary combination treatment. In that study, combination systemic/intramammary therapy with a combination penicillin/dihydrostreptomycin product was superior to 3 days of systemic therapy with penicillin and equivalent to 5 days of penicillin systemic treatment.

Clinical mastitis after extended therapy was reported in 2 studies (Roy and colleagues[14] and Deluyker and colleagues[15]). Roy and colleagues reported 4 cases (12.9%) of clinical mastitis of 31 cows treated. All cases were due to yeast IMI approximately 1 week after the end of the therapy. The authors in this study used sodium cephapirin 2 times a day for 5 days. Deluyker and colleagues reported an incidence of 5.2% of clinical mastitis in the group treated for 8 days with pirlimycin compared to 1.8% for the control group (pirlimycin for 2 days). These findings were also reported in other studies not keep in this systematic review[17,22] and constitute a potential drawback of extended therapy. Cow death following clinical mastitis after extended therapy using pirlimycin had been previously reported[17,22] but not in articles included in this systematic review.

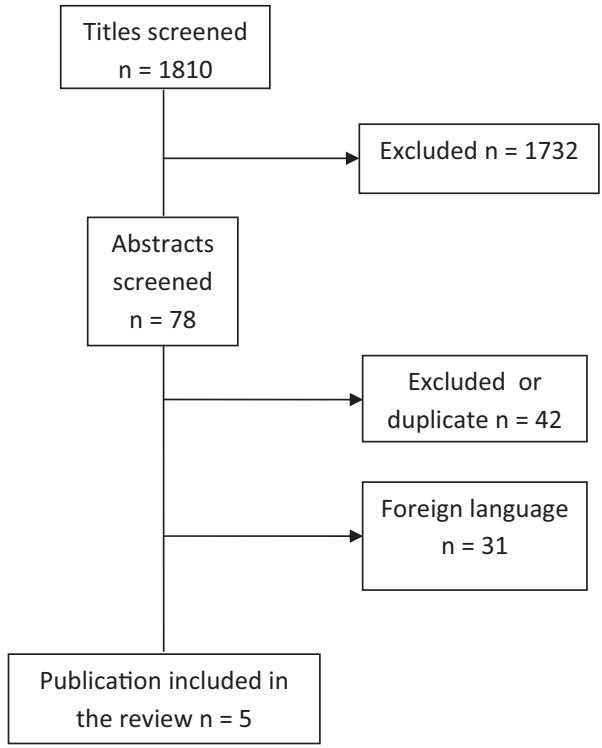

Fig. 3. Flow chart of selection process using CAB database (1973–2011 week 32).

In addition to treatment, Deluyker and colleagues[15] pretreatment SCC and quarter location (front vs back) were significantly associated with treatment response and older parity groups tended to have lower response to treatment.

DISCUSSION

This was the first time for both reviewers to conduct a systematic review using a scoring system. The authors are convinced that the process will increase validity of clinical decision-making and give a sound answer to the question asked.

However, some limitations on the process used should be reiterated before going further. Exclusion of publications was based on a rigorous application of the CONSORT 2010 checklist. Very few studies met the minimum criteria. No attempt was made to contact authors to add missing data to the published studies. Additionally, non–peer-reviewed publications were de facto excluded, as well as all non-English publications. Those decisions were made by the authors but could have been managed in a different way by others.

The 100-point scored version of the CONSORT 2010 checklist was used, (see article by Vandeweerd and colleagues elsewhere in this issue for further exploration of this topic) with a cut-off level for inclusion in the systematic review of 50 points. Consensus was apparent on 10 of 13 articles after initial review and agreement was reached on the final 3 articles after discussion between the authors. The majority of the articles lost points in several categories related to the material and methods

Table 1
Summary of study design features of 6 articles describing treatment efficacy during lactation against *S aureus* that met minimum selection criteria for inclusion in the systematic review

Author	Study Design	Number of Herds	*S aureus* Cases (n)	Treatment	Treatment Type	Controls
Roy et al[14]	Randomized clinical trial	14	61	Cephapirin sodium	5-day intramammary	Untreated
Deluyker et al[15]	Randomized clinical trial	54 (Study 1) 51 (Study 2)	140 122	Pirlimycin Pirlimycin	2-day intramammary 8-day intramammary	Untreated 2-day intramammary
Oliver et al[16]	Randomized clinical trial	3	50	Ceftiofur	2-, 5-, or 8-day intramammary	Untreated
Wilson et al[21]	Retrospective study[a]	Not specified	1272	Amoxicillin Cloxacillin Erythromycin Penicillin	Not described	Untreated
Wilson et al[9]	Randomized clinical trial	10	54	Florfenicol	3 intramammary treatments 12 hours apart	3 intramammary cloxacillin treatments 12 hours apart
Jarp et al[19]	Randomized clinical trial	Not specified	460	Penicillin	3 or 5 daily intramuscular treatments	1 intramuscular treatment of penicillin/dihydrostreptomycin followed by 4 daily intramammary treatments with a penicillin/ dihydrostreptomycin ointment

[a] Only antibiotic treatments with ≥20 cases are presented.

Table 2
Case definitions for enrolment and cure assessment for treatment efficacy against *S aureus*

Author	Case Definition	Cure Definition
Roy et al[14]	All cases subclinical Eligible if previous history of *S aureus* culture Enrolled if positive quarter culture on a minimum of 1 of 2 samples taken at 28 and 14 days prior to treatment	1. At quarter level, if *S aureus* was not recovered from previously infected quarters at 10, 24, and 31 days post treatment 2. At cow level, if all quarters were *S aureus* negative at 10, 24, and 31 days post treatment
Deluyker et al[15]	All cases subclinical Eligible if history of 2 consecutive SCC >250,000 or 1 >400,000 Enrolled if positive quarter culture and SCC >300,000 within 8 days of treatment	Negative for the pretreatment pathogen on quarter culture at 22–23 and 29–30 days post treatment
Oliver et al[16]	All cases subclinical Eligible if history of SCC >400,000 Enrolled if positive quarter culture on both of 2 samples taken 14 and 7 days prior to treatment	Negative for the pretreatment pathogen on quarter culture at 14 and 28 days post treatment
Wilson et al[21]	All cases subclinical Enrolled if positive quarter culture herd screening for which a second sample was recultured within 1 month	Negative for the pathogen identified by quarter herd screening on the subsequent sample taken within 1 month
Wilson et al[9]	**Subclinical cases** Eligible is previous history of *S aureus* culture Enrolled if positive quarter culture at time of enrollment **Clinical cases** Enrolled if bacterial pathogen present on both duplicate pretreatment milk samples from a cow with abnormal milk that did not have concurrent systemic signs	Same both subclinical and clinical Negative on quarter milk culture at days 14, 21, and 28 or negative on a minimum of 2 of these 3 samples with a SCC <300,000 on day 28
Jarp et al[19]	All cases clinical and subclinical cases coming from the same cows Cows in lactation 1, 2, or 3 and <6 months in lactation with abnormal secretion and/or visible signs of inflammation from which a penicillin-sensitive bacterium was cultured or quarters with no bacteria found but an increased SCC	Bacteriologic negative at 24–26 days and with low SCC for a quarter that had high SCC or a pathogen isolated on initial culture

Table 3
Summary of cure rates for treatment efficacy against *S aureus*

		Cure Rate (n)	
Author	**Treatment**	**Subclinical**	**Clinical**
Roy et al[14]	Negative control	3.3%[a] (1/30 cows)	NA
	5-day Intramammary Cephapirin sodium	25.8%[b] (8/31 cows)	NA
Deluyker et al[15]	Negative control	6 %[a] (4/63 quarters)[d]	NA
	2-day Intramammary Pirlimycin	56%[b] (82/146 quarters)[d]	NA
	8-day Intramammary Pirlimycin	86%[c] (46/53 quarters)[d]	NA
Oliver et al[16]	Negative control	0%[a] (0/12 quarters)	NA
	2-day ceftiofur	7%[a] (1/15 quarters)	
	5-day ceftiofur	17%[a,b] (2/12 quarters)	
	8-day ceftiofur	36%[b] (4/11 quarters)	
Wilson et al[21]	No treatment	43%[a] (471/1088 quarters)	NA
	Amoxicillin	43%[a] (30/70 quarters)	
Wilson et al[9]	Cloxacillin	47%[a] (23/49 quarters)	
	Erythromycin	65%[a] (15/23 quarters)	
	Penicillin	65%[a] (15/23 quarters)	17%[a] (2/12 quarters)
	Cloxacillin	6%[a] (1/17 quarters)	
	Florfenicol	0%[a] (0/14 quarters)	18%[a] (2/11 quarters)
Jarp et al[19]	Intramuscular penicillin/dihydrostreptomycin plus 4-day intramammary penicillin/dihydrostreptomycin	NA	40.6%[a] (54/133 quarters)
	3-day intramuscular penicillin		27.3%[b] (30/110 quarters)
	5-day intramuscular penicillin		46.8%[a] (59/126 quarters)

[a,b,c] Values within the same study with different superscripts are different ($P < .05$).
[d] Estimated based on models and sample size.

section, such as allocation ratio not presented, ethical protocol reference not presented, determination of the sample size not presented, and missing details about the random allocation (eg, methods used, by whom). Some points were also lost in other categories such as the introduction (eg, identification of a randomized trial in the title, presentation of the null hypothesis), the results section (eg, baseline demographic and clinical characteristics of each group, estimated effect size, and its precision), and the discussion (eg, trial limitations and source of potential bias, external validity), and the acknowledgment for the funding source of the research project was often not mentioned. Future articles should report more thoroughly the specific points just mentioned to allow a better comparison between studies. Researchers, reviewers, and editors should increase awareness of everyone involved in the process and make this issue a priority. Recently, guidelines to report trials and observational studies were published in veterinary journals.[23,24]

Direct comparison between studies has to be done with caution because of the large variation in study designs observed. One major variation observed is IMI and cure definitions. It is well established that milk bacteriology is not 100% sensitive. Dohoo and colleagues recently published a study that could be used to standardize these IMI definitions.[25] These definitions should be used in the future by researchers to report their work. That being said, some findings of this systematic review are valuable to discuss.

There is no evidence supporting combining systemic and local therapy for the control of *S. aureus* IMI during lactation. Only 1 study using that kind of approach was included in our systematic review.[19] More well-structured and -reported studies are needed in the future to evaluate this treatment regimen.

In general, cure rates for *S aureus* were low (<50%), with the exception of the rates of Deluyker and colleagues[15] and Wilson and colleagues.[21] Antibiotics used in those studies achieving higher cure rates were pirlimycin[15] and erythromycin or penicillin.[21] However, the latter study was a retrospective study and cure was assessed by only one milk culture approximately 30 days after clinical mastitis. Consequently, many factors could bias those results so they are less reliable. Considering this, pirlimycin seems to have the best cure rates among intramammary antibiotics. However, no direct comparison between antibiotics including pirlimycin is available so a definitive conclusion is impossible to make. Only Roy and colleagues reported cure rates at the cow level.[14] Decisions to attempt treatment versus cull for *S aureus* mastitis would typically be made at the cow rather than quarter level. Cure rates are inherently higher at the quarter level. Caution should be taken when extrapolating quarter level data to the cow-level decision.

Where duration of therapy with the same product was assessed, longer-duration therapy had a positive association with cure rate. This was done for pirlimycin[15] and ceftiofur.[16] Since all intramammary antibiotics are time-dependent antibiotics, it is logical that the longer the duration of the therapy, the better will be the results. Some drawbacks, like increased risk for clinical mastitis and treatment costs, should be considered and discussed with the producer before implementing such a treatment regimen.

The scope of this review was limited to antibiotic therapy. Cure rates for treatment depend on a number of cow and pathogen factors in addition to the therapeutic protocol. Cow factors include age, historical somatic cell count, duration of infection, and number of quarters infected. Pathogen factors include the numbers of colonies recoverable by culture and penicillin resistance. A full review of these risk factors was presented by Barkema and colleagues.[7]

SUMMARY

Based on this systematic review and considering available data, the best therapeutical option currently available in North America to treat *S aureus* IMI during lactation is an extended intramammary therapy for 5 to 8 days. Regarding specific antimicrobials, because direct comparison was not made among antibiotics and both enrollment and cure criteria at the cow and quarter level were inconsistent across studies, definitive conclusions are difficult. Pirlimycin seems to have higher cure rates at the quarter level than other studies reporting similar data and is labeled for extended therapy in both the United States and Canada. Caution should be exercised because several studies reported a spike in clinical mastitis rates following extended therapy. There is no evidence that a systemic antibiotic treatment should be combined to increase cure rates. More research is needed to validate those findings. Improvement is needed in research protocols including definition of IMI and cure and the publication process to facilitate evaluation and comparison between studies.

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