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# Critically important antimicrobials are generally not needed to treat nonsevere clinical mastitis in lactating dairy cows: Results from a network meta-analysis

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## ABSTRACT

There is ongoing debate regarding whether critically important antimicrobials (CIA) should be used to treat infections in food-producing animals. In this systematic review, we determined whether CIA and non-CIA have comparable efficacy to treat nonsevere bovine clinical mastitis caused by the most commonly reported bacteria that cause mastitis worldwide. We screened CAB Abstracts, Web of Science, MEDLINE, Scopus, and PubMed for original epidemiological studies that assessed pathogen-specific bacteriological cure rates of antimicrobials used to treat nonsevere clinical mastitis in lactating dairy cows. Network models were fit using risk ratios of bacteriological cure as outcome. A total of 30 studies met inclusion criteria. Comparisons of cure rates demonstrated that CIA and non-CIA had comparable efficacy for treatment of nonsevere clinical mastitis in dairy cattle. Additionally, for cows with nonsevere clinical mastitis caused by *Escherichia coli* and *Klebsiella* spp., bacteriological cure rates were comparable for treated versus untreated cows; therefore, there was no evidence to justify treatment of these cases with CIA. Our findings supported that CIA in general are not necessary for treating nonsevere clinical mastitis in dairy cattle, the disease that accounts for the majority of antimicrobial usage in dairy herds worldwide. Furthermore, our findings support initiatives to reduce or eliminate use of CIA in dairy herds.

**Key words:** critically important antimicrobial, dairy cow, mastitis, treatment

## INTRODUCTION

Antimicrobial resistance is one of the most important global threats to human and animal health. It is estimated that without urgent action, we are heading toward a postantibiotic era where 10 million deaths per year globally will be attributable to antimicrobial resistance (O'Neill, 2016). It is recognized that antimicrobial use contributes to emergence of antimicrobial resistance (Chantziaras et al., 2014); therefore, antimicrobial use should be refined. The World Health Organization (**WHO**) has promulgated a set of strategies to combat antimicrobial resistance, including reduction of antimicrobial use in food-producing animals.

The WHO classifies antimicrobials into categories based on availability of alternatives and risk of antimicrobial resistance–emergence due to nonhuman antimicrobial use (WHO, 2016). Critically important antimicrobials (**CIA**) are those used to treat specific diseases in humans, including infections acquired from nonhuman sources. The use of CIA in food-producing animals may be associated with increased risk of nontreatable human infections (Dutil et al., 2010). Hence, there is an ongoing debate on whether these antimicrobials should be used to treat infections in food-producing animals (Collignon, 2013). In the absence of definitive answers, several countries have directed substantial efforts to control overall use of CIA in food-producing animals, either by promoting antimicrobial stewardship or by restricting their use (Dupont et al., 2017). Ideally, alternatives of comparable or superior efficacy should be available as part of any stewardship initiative to reduce use of CIA in food-producing animals. Such alternatives would allow for effective treatment of bacterial infections that negatively affect animal health and welfare, while minimizing their effect on efficacy of CIA for human medicine.

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It is unclear whether CIA are indispensable for treating infections in food-producing animals; an evidence-based assessment would rely on comparisons of efficacy of CIA versus non-CIA. Mastitis accounts for most antimicrobial usage, including CIA, in dairy cattle (Nobrega et al., 2017). Despite numerous randomized clinical trials comparing efficacy of various antimicrobials for treating mastitis (Schukken et al., 2013), approximately all studies assessed only 1 or 2 antimicrobial treatment protocols or antimicrobial classes, thereby falling short to assess the need of CIA to treat mastitis. However, network meta-analysis, a natural extension of meta-analysis (Tonin et al., 2017), efficiently handles multiple treatment protocols and facilitates comparison of results from multiple trials in a single analysis by integrating direct and indirect evidence (e.g., indirect comparison of distinct interventions compared with a same control condition in separate studies).

In this systematic review, we assessed whether CIA and non-CIA have comparable efficacy to treat nonsevere bovine clinical mastitis (**CM**) caused by the most commonly isolated bacterial pathogens worldwide. We used a set of networks to analyze literature reporting on bacteriological cure rates of antimicrobials used to treat lactating dairy cows with nonsevere CM.

## MATERIALS AND METHODS

### Eligibility Criteria

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) network meta-analysis reporting standards (Hutton et al., 2015). We did not publish the following review protocol before conducting the study. Epidemiological studies (observational or experimental) that assessed pathogen-specific bacteriological cure rates of antimicrobials for treating nonsevere CM in lactating dairy cows were eligible for inclusion. Nonsevere CM was defined as signs of inflammation in the mammary gland or altered milk secretion with no signs of systemic involvement. The CM cases without any mention of location and severity of inflammation signs were considered nonsevere because that is the most common presentation of CM in dairy herds (Swinkels et al., 2013b; Kalmus et al., 2014). Intervention was defined as administration of antimicrobials, either systemically or locally (intramammarily). We required inclusion of a comparator group defined as follows: (1) animals with nonsevere CM caused by same pathogen or pathogen group and (2) animals under a different antimicrobial treatment protocol (e.g., different molecule, dose, route of administration, or days under treatment) or untreated. Animals were considered untreated if they received no therapy of any kind or treated with placebo. Consequently, dairy cows that were frequently milked, or that received oxytocin or nonsteroidal anti-inflammatory drugs (**NSAID**) solely, were not considered untreated. Furthermore, studies evaluating alternative therapies (e.g., frequent milking, NSAID, homeopathy) in addition to antimicrobial treatments were excluded in absence of a second group with a distinct antimicrobial treatment protocol or an untreated group. Studies where antimicrobial treatments were performed following antimicrobial susceptibility testing were not considered unless there were at least 2 treatment protocols in same population stratified by resistance phenotype status (e.g., cows with CM caused by resistant *S. aureus* under 2 distinct treatment protocols). Bacteriological cure, defined as failure to isolate or detect the same species detected before treatment onset, was considered as the outcome. It was measured at the cow- or quarter-level, outside the withdrawal period but within the first month after last treatment day, using either single or multiple milk samplings. Thus, studies reporting exclusively on clinical cure or other metrics of cure (e.g., improvement, any pathogen cure) were not considered. Milk samples collected within an interval (e.g., 19–41 d) were assumed as collected at the window mean (e.g., 30 d for the 19–41-d window). Finally, studies only reporting cure rates without any mention of etiological agent were not eligible for inclusion.

### Search Strategy and Study Selection

We screened CAB Abstracts, Web of Science (all databases), MEDLINE, Scopus (Elsevier), and PubMed on January 30, 2019 for potentially relevant articles. We developed a search strategy consisting of relevant key words describing the 3 following themes: population, intervention, and outcome. The broad themes were combined into a single query. Although we did not place a limit on publication date and language during our initial screening, English, Spanish, Portuguese, French, and Italian articles published from 1980 onwards were considered for full-text reviewing. The initial search strategy was designed to be fairly broad. Preliminary assessment of returned hits indicated a substantial number of studies in species other than dairy cattle. Additionally, review articles, case reports, and case series where no comparator was used were also retained by our initial search strategy. Therefore, our search strategy was further refined to exclude review articles and case reports in addition to noncattle studies using the Boolean operator "NOT" at title level

(Supplemental Text S1, https://doi.org/10.3168/jds.2020-18365). Queries were adapted to database-specific terms, as necessary.

Our search strategy was enhanced by screening relevant conference proceedings using the following 3 specialized databases: (1) Searchable Proceedings of Animal Conferences (SPAC, 2019), (2) National Mastitis Council Proceedings Library (NMC, 2019), and (3) International Veterinary Information Service (IVIS, 2019). The query "mastitis treatment" was used in all 3 databases, and titles from all hits were reviewed. Additionally, abstracts from the third International Mastitis Seminar (IDF et al., 1995; Tel Aviv, Israel) were manually reviewed. Finally, reference lists from all articles included in this systematic review were reviewed for potential inclusion.

Two authors (D. N. and S. A. N.) reviewed all titles and abstracts independently. Original research that reported on efficacy of antimicrobials to treat dairy cows with mastitis during lactation were retained. This initial screening was fairly broad to encompass all potentially relevant studies for full-text review. Discrepancies between reviewers were resolved by consulting with a third reviewer (H. B.). Agreement between reviewers was excellent ($\kappa = 0.92$; 95% CI: 0.85–0.99). All full texts were retrieved and assessed for eligibility using a pre-established screening tool. Epidemiological studies that reported on bacteriological cure rates of $\geq$2 distinct antimicrobial treatment protocols (or 1 protocol and an untreated group) for treating dairy cows with CM during lactation were retained, according to previously stated eligibility criteria.

### Data Collection

The same reviewers (D. N. and S. A. N.) extracted data independently from individual studies using an electronic form in Microsoft Excel (Redmond, WA). Variables extracted, when available, were author, year, country, study design, CM definition, number of cows and quarters with CM, eligibility criteria for animals included in the study, bacteria, bacteria identification method, antimicrobial treatment, route of administration, dose, days under treatment, type of supportive therapy, time of outcome measurement, level of outcome measurement (i.e., cow or quarter), outcome definition, and numbers of cases (cows or quarters) and cures per treatment protocol. Lastly, authors from studies that had missing required information were contacted by e-mail with regards to availability of data.

For studies in which posttreatment samples were collected on multiple days and cure was assessed individually for each sampling, results from sampling closest to median time of outcome measurement for CM caused by the same pathogen were selected for extraction. If studies reported nonequivalent cure rates at quarter- and cow-level, only the former were retained.

### Risk of Bias and Study Quality

The same 2 reviewers independently assessed quality and risk of bias in individual experimental studies using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool (Hooijmans et al., 2014). Risk of bias was classified according to number of error sources that each study had failed to address: low risk of bias (1–3 sources of error), moderate risk of bias (4–6 sources of error), and high risk of bias (7–9 sources of error). A single experimental study classified as having high risk of bias, as well as nonexperimental studies, had their effect on final estimates assessed using sensitivity analysis.

### Data Synthesis and Network Meta-Analyses

Prior to analyses, categories of antimicrobial treatment protocols, supportive therapy, and pathogens were generated. Antimicrobial treatment protocols were grouped using categories defined a priori based on the WHO fifth revision of Critically Important Antimicrobials for Human Medicine (WHO, 2016) and route of administration (systemic and intramammary). The WHO categories included newer generation (third, fourth, and fifth) cephalosporins, macrolides, quinolones, penicillins (natural), amphenicols, penicillins (anti-staphylococcal), and older generation (first and second) cephalosporins. For combinations containing 1 or more antimicrobial or different classes given by different routes, the prioritization category of each molecule was initially determined and used thereafter to classify protocols as follows: (1) combination containing 1 or more highest-priority antimicrobial class or (2) combination containing 1 or more high-priority antimicrobial class. In the few trials where antimicrobials were administered by more than 1 route, protocols were classified as systemic treatments if 1 or more antimicrobial was administered parenterally; their effect on final estimates was assessed through sensitivity analysis, as described. When studies compared effects of routes of administration using systemic, intramammary, and both routes of administration concurrently, results from individual routes were retained exclusively. Finally, because extended therapies are known to affect the probability of bacteriological cure using some antimicrobials (Oliver et al., 2004), and a wide range of days under treatment with cephalosporins was reported (from 1–8), a cut-off of 4 d was used to define an extended therapy with cephalosporins. For other antimi-

crobial classes, no significant variations were detected in terms of days under treatment (Supplemental Table S1, https://doi.org/10.3168/jds.2020-18365).

Despite reported lack of efficacy of nonantimicrobial approaches for treating CM (Francoz et al., 2017), use of supportive therapy may affect efficacy of antimicrobials (Roberson et al., 2004). Within a study, each treatment group had its supportive therapy classified as: (1) no use of supportive therapy, (2) intramammary anti-inflammatories (e.g., formulation containing prednisolone), (3) systemic anti-inflammatories (e.g., NSAID), and (4) frequent milking (with or without oxytocin administration). For studies reporting cure rates from multiple antimicrobial treatment protocols where a subset differed on use of supportive therapies exclusively, results from groups receiving supportive therapy were not considered. To be as comprehensive as possible while ensuring a manageable number of treatment categories, use of supportive therapy was handled through sensitivity analyses in network analyses, as described.

Culture results were classified as undetermined (not cultured), *Escherichia coli*, *Klebsiella* spp., other coliforms (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., or any combination of these bacteria), unspecified coliforms, non-agalactiae streptococci (*Streptococcus uberis*, *Streptococcus dysgalactiae*, non-*agalactiae* streptococci, or any combination of these bacteria), non-*aureus* staphylococci (coagulase-negative staphylococci, *Staphylococcus* spp. other than *Staphylococcus aureus*), *S. agalactiae*, *S. aureus*, no growth, and others (*Bacillus* spp., *Pseudomonas* spp., *Trueperella pyogenes*, *Nocardia* spp., yeasts, molds, *Prototheca* spp., *Enterococcus* spp., *Corynebacterium* spp., *Pasteurella* spp., *Proteus* spp.). For any combination of isolates obtained in a single culture, except those described under coliforms and non-*agalactiae* streptococci, results were classified as mixed culture. However, for studies reporting individual results for each bacterial species, irrespective of presence of a second species in culturing, results from mixed cultures were grouped into 2 pathogen categories, accordingly. Lastly, grouped results excluding those described (e.g., major or minor pathogen, gram-positive or negative) were classified as combined.

Five network meta-analyses were carried out according to the most commonly reported bacteria that cause CM worldwide (*S. aureus*, *E. coli*, non-*aureus* staphylococci, non-*agalactiae* streptococci, and *Klebsiella* spp.). Analyses were carried out in R version 3.5.2 using the "netmeta" package (Rücker et al., 2019). Statistical significance was considered at the 5% level.

A study was eligible for inclusion in networks if: (1) it reported specific rates of bacteriological cure of nonsevere CM caused by aforementioned bacteria, (2) it had $\geq$2 categories of antimicrobial treatments per pathogen after grouping, and (3) 1 or more of these categories was common to >1 study included in the pathogen-specific network. If a study provided multiple estimates within the same grouping (e.g., 2 treatment protocols or distinct bacterial species receiving same classification), results were first merged accordingly before assessment of eligibility for inclusion in networks. For within-study pairwise comparisons where no cure was reported for both protocols (probability of cure equal to 0 for 2 protocols within a study), results from the most uncommon protocol were dropped from the analyses. Studies not eligible for inclusion in any network had their results discussed qualitatively.

A frequentist approach using generalized linear models applied using graph theory was used to develop networks (Rücker, 2012). All treatment networks analyzed were closed, as our eligibility criteria for inclusion of a study in networks required common treatment arms between studies. Models were fit using risk ratios for bacteriological cure as the outcome, and random effects to allow for unmeasured, between-study differences. Network inconsistency (a measure of agreement between direct and indirect evidence comparing the same treatments) was assessed using the Q statistic, a measure of discrepancy between the observed risk ratios for a given comparison, compared with what is expected based on any indirect comparisons (Rücker, 2012). Inconsistency was also visually assessed to identify particular studies or comparisons contributing to the inconsistency based on heatmaps generated using a function within "*netmeta*" (Rücker et al., 2019). The proportion of indirect to direct evidence was also assessed in each model, providing an indication of how many uncommon treatments (present in only a single study) were evaluated and combined with confidence intervals (CI) to assess strength and validity of the findings. When inconsistency was detected, a sensitivity analysis (described below) was conducted to determine the effect of the study's contribution to inconsistency on the overall model fit. Studies removed from the quantitative network meta-analysis were then described qualitatively. Model heterogeneity was assessed using the $I^2$ statistic computed with the random-effects model in "*netmeta*" (Rücker et al., 2019), representing the ratio of between-study variance to within-study variance. A significant $I^2$ value indicated that unmeasured study characteristics contributed to observed differences more than the treatments themselves. We evaluated all pairwise comparisons of CIA versus each non-CIA using league tables. A CIA category was deemed to be different from non-CIA if relative risks and respective 95% CI comparing bacteriological cure rates between categories did not include the null value for each pairwise comparison.

Sensitivity analyses were used to evaluate effects of categories of supportive therapy in our estimates. Treatment protocols containing a specific type of supportive therapy were removed 1-at-a-time, 2-at-a-time (intramammary anti-inflammatory and frequent milking exclusively), and all 3 at once. Relative risks and respective 95% CI, heterogeneity, and inconsistency estimates were compared from a different set of models for each pathogen. Choice of models was based on (1) consistency of findings when compared with models containing only treatment protocols without supportive therapy, (2) lowest heterogeneity (using a threshold of 20 percentage points to infer similarity) and absence of statistical significance for inconsistency estimates, and (3) highest degree of network completeness (defined as the highest possible number of treatment arms or nodes) and studies included while meeting criteria 1 and 2. Finally, a secondary set of sensitivity analyses was carried out 1-at-a-time on selected models to explore potential sources of inconsistencies detected, unusual routes of administration, high risk of bias, nonexperimental evidence, effects of combined route therapies, and pathogen antimicrobial resistance profile. Major findings were robust regardless of presence of refrained studies; choice of models was, therefore, based on heterogeneity and inconsistency estimates using same criteria aforementioned. We did not attempt any additional meta-regression or subgroup analyses for our final models.

A second antimicrobial classification scheme was used to assess the robustness of findings from initial networks. This secondary assessment was done to ensure that very distinct protocols classified as "combinations" in our first assessment did not significantly affect our overall findings, despite low inconsistency and heterogeneity values detected in our initial models. In this secondary scheme, combinations containing one or more antimicrobial were first classified as (1) combination containing aminoglycosides and (2) combination containing anti-staphylococcal penicillins, and analyses were carried out as described. A single study comparing 2 combination protocols that would not fall under the prespecified categories (Sol et al., 2000) was excluded from this secondary assessment. Because findings were robust irrespective of classification scheme, results from initial approach were reported exclusively.

Bias assessment at the reporting level was evaluated using comparison-adjusted funnel plots (Chaimani and Salanti, 2012), where estimated relative risk from random-effects model for specific treatment comparisons were plotted against their respective standard errors. Asymmetry was tested using both Egger's regression test and the Thompson-Sharp regression test, allowing for between-study heterogeneity.

## RESULTS

### Description of Studies

Our search strategy yielded 9,173 records (Figure 1), from which 30 studies were retained (Aguilera, 1983; Guterbock et al., 1993; Lavy et al., 1995; Wilson et al., 1996; Shpigel et al., 1997; McDougall, 1998; Pyorala and Pyorala, 1998; Roberson, 1998; Sol et al., 2000; Hillerton and Kliem, 2002; McDougall, 2003; Taponen et al., 2003a,b; Wraight, 2003; Oliver et al., 2004; Roberson et al., 2004; Sérieys et al., 2005; McDougall et al., 2007a, b; Bradley and Green, 2009; Schukken et al., 2011; Schukken et al., 2013; Swinkels et al., 2013a; Kalmus et al., 2014; Swinkels et al., 2014; Truchetti et al., 2014; Bryan et al., 2016; Cortinhas et al., 2016; Vasquez et al., 2016; Tomazi et al., 2018). Twenty-six studies were included in 1 or more network. Reasons for noninclusion in any network were as follows: (1) flagged as source of inconsistency assessed using the Q statistic and visual inspection of heatmaps (Schukken et al., 2011), (2) high risk of bias (Aguilera, 1983), and (3) absence of $\geq$2 antimicrobial treatment protocols in different categories following treatment classification (Lavy et al., 1995; McDougall, 2003).

Out of 30 studies, 25 were randomized clinical trials (Table 1). The majority of studies defined CM by using signs of local inflammation and characteristics of milk secretion. Total number of cows enrolled per study ranged from 23 to 1,462. Most studies (28 of 30) reported quarter-level or equivalent cow- and quarter-level results (e.g., enrollment of single quarter per cow). Only 3 studies used molecular diagnostic techniques for identification of bacteria that cause CM (McDougall et al., 2007a; Schukken et al., 2011; Kalmus et al., 2014).

Most treatment protocols relied on use of 1 or more intramammary antimicrobials (Table 2); number of treatment protocols within a study ranged from 2 to 7. The CIA of high priority were the most frequently used antimicrobials. Among non-CIA, protocols using first and second generation cephalosporins and anti-staphylococcal penicillins were most often implemented. The most frequently studied pathogen was *S. aureus* (22 studies), whereas relatively few (n = 8) studies reported on bacteriological cure of *Klebsiella* spp.

### Escherichia coli

Fourteen studies reporting on bacteriological cure rates of antimicrobials used to treat nonsevere CM caused by *E. coli* were eligible for inclusion in networks. From this total, 1 (Schukken et al., 2011) was flagged as a potential source of inconsistency and therefore excluded (Supplemental Table S1, https://doi.org/10

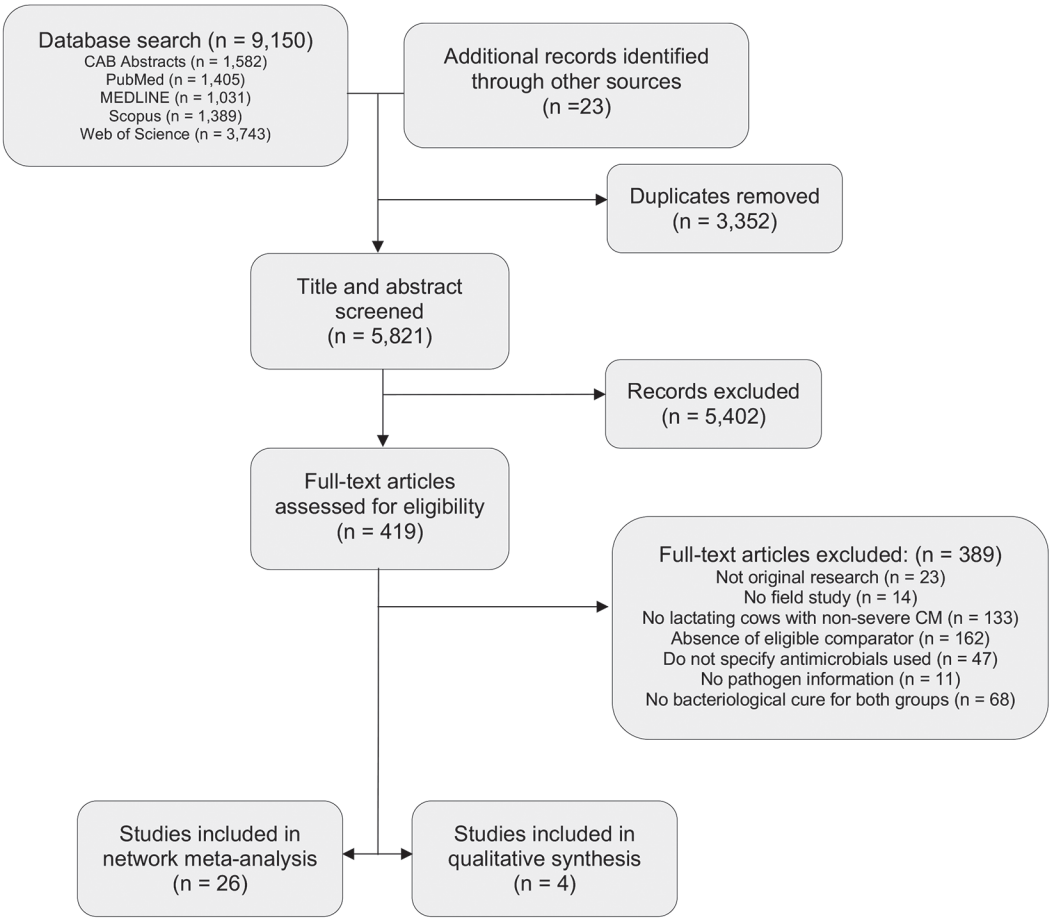


**Figure 1.** Flow diagram of the study selection process.

.3168/jds.2020-18365). Nevertheless, findings were consistent, irrespective of inclusion of this study; there was no evidence supporting the need of CIA for treating nonsevere CM caused by *E. coli* (Figure 2). No protocol including the use of CIA had superior bacteriological cure rates of nonsevere *E. coli* CM than protocols relying on non-CIA (Supplemental Table S2, https://doi.org/10.3168/jds.2020-18365). Additionally, there was no evidence to support use of antimicrobials for treating nonsevere CM caused by *E. coli*, as the probability of bacteriological cure was similar for treated versus untreated cows (Figure 2).

### *Klebsiella spp.*

From the 8 studies reporting on bacteriological cure rates of antimicrobials for treating nonsevere CM caused by *Klebsiella* spp., 7 were eligible for inclusion in networks. From this total, a single study (Schukken et al., 2011) was excluded because it was a significant source of heterogeneity and inconsistency (Supplemental Table S3, https://doi.org/10.3168/jds.2020-18365). There was no evidence supporting the need to use CIA for treating nonsevere CM caused by *Klebsiella* spp. (Figure 3, Supplemental Table S4, https://doi.org/10.3168/jds.2020-18365), despite inclusion of the potential source of inconsistency in analysis: no protocol including the use of CIA for treatment of nonsevere CM caused by *Klebsiella* spp. resulted in increased bacteriological cure compared with protocols including non-CIA (Supplemental Table S4). Extended treatment with third generation cephalosporins, antimicrobials classified as CIA, was not superior to protocols based on use of first generation cephalosporins, a non-CIA antimicrobial (relative risk = 1.67, 95% CI: 0.87–3.20; Supplemental Table S4). In addition, probability of bacteriological cure was not lower when no antimicrobials were administered (Figure 3), as reported from 2 trials. Nevertheless, the relatively low sample size of studies involved warrants further investigation.

**Table 1.** Summary of study characteristics

| Reference | Country | Design[1] | Cows (quarters)[2] | CM definition[3] | | Pathogen ID[4] | | Interval to outcome[5] (d) | Outcome level |
|---|---|---|---|---|---|---|---|---|---|
| | | | | IF | MS | Cult | Mol | | |
| Aguilera, 1983 | Cuba | CT | 250 (503) | | ● | ● | | 11–14 | Quarter |
| Bradley and Green, 2009 | UK, France, Germany | RCT | 491 (491) | ● | ● | ● | | 16, 25 | Quarter |
| Bryan et al., 2016 | New Zealand | RCT | 458 (458) | ● | ● | Not specified | | 9,16, 23 | Quarter |
| Cortinhas et al., 2016 | Brazil | RCT | 358 (358) | ● | ● | ● | | 14, 21 | Quarter |
| Guterbock et al., 1993 | USA | RCT | NS (254) | ● | ● | ● | | 9, 11, 21 | Quarter |
| Hillerton and Kliem, 2002 | UK | RCT[6] | 54 (72) | ● | ● | ● | | 14, 21[7] | Quarter |
| Kalmus et al., 2014 | Estonia | RCT | 140 (140) | ● | ● | | ● | 21–28 | Quarter |
| Lavy et al., 1995 | Israel | RCT | 26 (52) | ● | ● | Not specified | | 15 | Quarter |
| McDougall, 1998 | New Zealand | RCT | NS (798) | ● | ● | ● | | 14, 21 | Quarter |
| McDougall, 2003 | New Zealand | RCT | 282 (404) | ● | ● | ● | | 19–22 | Quarter |
| McDougall et al., 2007a | New Zealand | RCT | 1,070 (1,342) | ● | ● | ● | ● | 14, 21 | Quarter |
| McDougall et al., 2007b | New Zealand | RCT | 1,462 (1,561) | ● | ● | ● | | 21–42 | Quarter |
| Oliver et al., 2004 | USA | RCT | 23 (37) | ● | ● | ● | | 7, 14, 21, 28[7] | Quarter |
| Pyorala and Pyorala, 1998 | Finland | RC | 487 (543) | Not specified | | ● | | 21–28[7] | Quarter |
| Roberson, 1998 | USA | ND | 54 (NS) | ● | ● | ● | | 8, 15, 22, 29, 36 | Cow |
| Roberson et al., 2004 | USA | RCT | 70 (99) | ● | ● | ● | | 8, 15, 22, 29, 36 | Cow |
| Schukken et al., 2011 | USA | RCT | 104 (104) | ● | ● | ● | ● | 7, 14[7] | Quarter |
| Schukken et al., 2013 | USA | RCT | 321 (321) | ● | ● | ● | | 10, 17 | Quarter |
| Sérieys et al., 2005 | France | RCT | 312 (312) | ● | ● | ● | | 17, 22 | Quarter |
| Shpigel et al., 1997 | Israel | RCT | 47 (94) | ● | ● | ● | | 7, 14[7] | Quarter |
| Sol et al., 2000 | The Netherlands | RC | 958 (958) | ● | ● | ● | | 14[7] | Quarter |
| Swinkels et al., 2013 | France, Hungary, Italy, the Netherlands, UK | RCT | 1,217 (1,217) | ● | ● | ● | | 14–17, 21–27 | Quarter |
| Swinkels et al., 2014 | Germany | RCT | 435 (435) | ● | ● | ● | | 14, 21 | Quarter |
| Taponen et al., 2003a | Finland | RCT | 96 (117) | ● | ● | ● | | 21–28 | Quarter |
| Taponen et al., 2003b | Finland | RC | 118 (166) | ● | ● | ● | | 14, 28 | Quarter |
| Tomazi et al., 2018 | Brazil | RCT | 346 (346) | ● | ● | ● | | 14, 21 | Quarter |
| Truchetti et al., 2014 | Canada | RCT | 241 (241) | ● | ● | ● | | 7, 14, 21[7] | Quarter |
| Vasquez et al., 2016 | USA | RCT | 1,168 (1,168) | ● | ● | ● | | 14, 21 | Quarter |
| Wilson et al., 1996 | USA | RCT | NS (119) | ● | ● | ● | | 14, 21, 28[7] | Quarter |
| Wraight, 2003 | New Zealand | RCT | NS (440) | ● | ● | ● | | 10–11[7] | Quarter |

[1]Study design: RCT = randomized-controlled trial; CT = controlled trial; RC = retrospective cohort; ND = not determined.

[2]Number of cows with clinical mastitis (and quarters) initially enrolled.

[3]Clinical mastitis definition based on presence of inflammation signs (IF) or milk secretion alteration (MS).

[4]Pathogen identification method based on bacteriological culturing and biochemical testing (Cult) or molecular diagnosis (Mol).

[5]Interval to outcome (days) after enrollment in the study.

[6]Work conducted in 3 stages; 1 was an RCT.

[7]Interval to outcome in days after last treatment.

**Table 2.** Summary of findings from individual studies

| Reference | Pathogen[1] | | | | | | Treatment protocol (days under treatment, total number of treatments)[2] | Route[3] | | | Supportive therapy[4] | | | | Category[5] | | | Findings[6] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* | *Klebsiella* spp. | *Staphylococcus aureus* | NAS | Nag. strep. | Other | | Intramammary | Systemic | Combined | None | IMM AIM | Systemic AIM | FM | HSTP (CIA) | HP (CIA) | HI | |
| Aguilera, 1983 | | | ● | | | ● | 1- Penicillin 500,000 IU + streptomycin 500 mg (4 d, 4×) | ● | | | ● | | | | | ● | | 2 > 1 |
| | | | | | | | 2- Penicillin 10,000 IU/kg + streptomycin 5mg/kg of BW (4 d, 4×) | | ● | | ● | | | | | ● | | |
| Bradley and Green, 2009 | ● | | ● | ● | ● | ● | 1- Cefalexin 200 mg + kanamycin 133 mg (2d, 2×) | ● | | | ● | | | | | ● | | 1 = 2 > 3 |
| | | | | | | | 2- Cefquinome 75 mg (1.5 d, 3×) | ● | | | ● | | | | ● | | | |
| | | | | | | | 3- Cefoperazone 100 mg (2 d, 2×) | ● | | | ● | | | | ● | | | |
| Bryan et al., 2016 | | | ● | ● | ● | ● | 1- Penicillin 1 g + cloxacillin 200 mg (4 d, 4×) | ● | | | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- Oxytetracycline 200 mg + oleandomycin 100 mg + neomycin 100 mg (4 d, 4×) | ● | | | | ● | | | ● | | | |
| Cortinhas et al., 2016 | ● | ● | ● | ● | ● | ● | 1- Tetracycline 200 mg + neomycin 250 mg + bacitracin 28 mg (4d, 4×) | ● | | | | ● | | | | ● | | 1 = 2 |
| | | | | | | | 2- Ceftiofur 125mg (4 d, 4×) | ● | | | | | ● | | ● | | | |
| Guterbock et al., 1993 | | | | | ● | ● | 1- Oxytocin 100 IU (1.5 d, 3×) | | | | | | | | | | | 1 = 2 = 3 |
| | | | | | | | 2- Cephapirin 200 mg (1 d, 2×) | ● | | | ● | | | | | | ● | |
| | | | | | | | 3- Amoxicillin 62.5 mg (1.5 d, 3×) | ● | | | ● | | | | | ● | | |
| Hillerton and Kliem, 2002 | | | | | ● | | 1- Untreated | | | | | | | | | | | 5 = 6 = 7 > 1; 3 > 2 = 4[11] |
| | | | | | | | 2- Oxytocin 100 IU (3 d, 6×)[7] | | | | | | | | | | | |
| | | | | | | | 3- Penethamate hydriodide 150 mg + dihydrostreptomycin 150 mg + framycetin sulfate 50 mg (3 d, 3×) | ● | | | | ● | | | | ● | | |
| | | | | | | | 4- 2 and 3 | ● | | | | ● | | | | ● | | |
| | | | | | | | 5- Penethamate hydriodide 150 mg + dihydrostreptomycin 150 mg + framycetin sulfate 50 mg (3 d, 6×) | ● | | | | ● | | | | ● | | |
| | | | | | | | 6- Procaine penicillin 200 mg/25 kg + dyhidrostreptomycin 250 mg/25 kg (3 d, 3×) | | ● | | ● | | | | | ● | | |
| | | | | | | | 7- [5] + [6] | | | ● | | ● | | | | ● | | |
| Kalmus et al., 2014 | | | ● | ● | ● | ● | 1- Benzylpenicillin procaine 600 mg (5 d, 5×) | ● | | | | | ● | | | ● | | 1 = 2 |
| | | | | | | | 2- Benzylpenicillin procaine 20 mg/kg of BW (5 d, 5×) | | ● | | | | ● | | | ● | | |
| Lavy et al., 1995 | ● | | | | | | 1- Florfenicol 750 mg (1 d, 3×) | ● | | | ● | | | | | | ● | 1 = 2 = 3 |
| | | | | | | | 2- Florfenicol 1000mg (1d, 3×) | ● | | | ● | | | | | | ● | |
| | | | | | | | 3- Florfenicol 1250mg (1d, 3×) | ● | | | ● | | | | | | ● | |
| McDougall, 1998 | | | ● | ● | ● | ● | 1- Penethamate hydriodide (5 g) (1.5 d, 3×)[8] | | ● | | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- Penicillin procaine 1 g + dihydrostreptomycin 0.5 g (1.5 d, 3×) | ● | | | ● | | | | | ● | | |
| McDougall, 2003 | ● | | ● | ● | ● | ● | 1- Lincomycin hydrochloride 330 mg + neomycin sulfate 100 mg (1.5 d, 3×) | ● | | | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- Penicillin procaine 1 g + dihydrostreptomycin 0.5 g (1.5 d, 3×) | ● | | | ● | | | | | ● | | |
| McDougall et al., 2007a | | | ● | ● | ● | ● | 1- Penethamate hydroiodide 5 g (3 d, 3×) | | ● | | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- Tylosin 5 g (3 d, 3×) | | ● | | ● | | | | ● | | | |
| McDougall et al., 2007b | | | ● | ● | ● | ● | 1- Cefuroxime sodium 250 mg (1.5 d, 3×) | ● | | | ● | | | | | | ● | 1 = 2 = 3 |
| | | | | | | | 2- Penicillin procaine 1 g (1.5 d, 3×) | ● | | | ● | | | | | ● | | |
| | | | | | | | 3- Penicillin procaine 1 g + dihydrostreptomycin 0.5 g (1.5 d, 3×) | ● | | | ● | | | | | ● | | |

*Continued*

**Table 2 (Continued).** Summary of findings from individual studies

| Reference | Pathogen[1] | | | | | | Treatment protocol (days under treatment, total number of treatments)[2] | Route[3] | | | Supportive therapy[4] | | | | Category[5] | | | Findings[6] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* | *Klebsiella* spp. | *Staphylococcus aureus* | NAS | Nag. strep. | Other | | Intramammary | Systemic | Combined | None | IMM AIM | Systemic AIM | FM | HSTP (CIA) | HP (CIA) | HI | |
| Oliver et al., 2004 | | | | | ● | | 1- Ceftiofur 125 mg (2 d, 2×) | ● | | | ● | | | | ● | | | 2 = 3 > 1 |
| | | | | | | | 2- Ceftiofur 125 mg (5d, 5x) | ● | | | ● | | | | ● | | | |
| | | | | | | | 3- Ceftiofur 125 mg (8 d, 8×) | ● | | | ● | | | | ● | | | |
| Pyorala and Pyorala, 1998 | ● | | ● | ● | ● | | 1- Penicillin procaine 20 mg/kg of BW (3–5 d, SID) | | ● | | | | | ● | | ● | | 1 > 2 = 3 (for *S. aureus*) |
| | | | | | | | 2- Enroflixacin 2.5–5 mg/kg of BW (3–5 d, SID) | | ● | | | | | ● | ● | | | |
| | | | | | | | 3- Spiramycin 10 mg/kg of BW (3–5 d, SID) | | ● | | | | | ● | ● | | | |
| Roberson, 1998 | ● | ● | | | ● | ● | 1- Untreated | | | | | | | | | | | 2 > 1 (gram-positive); 1 = 2 (gram-negative) |
| | | | | | | | 2- Amoxicillin (unspecified) | ● | | | ● | | | | | ● | | |
| Roberson et al., 2004 | ● | ● | | | ● | ● | 1- Untreated | | | | | | | | | | | 3 > 1 = 2 = 3 (Nag. strep.); 1 = 2 = 3 = 4 (coliforms) |
| | | | | | | | 2- Frequent milking | | | | | | | ● | | | | |
| | | | | | | | 3- Amoxicillin 62.5 mg (2 d, 3×) | ● | | | ● | | | | | ● | | |
| | | | | | | | 4- 2 + 3 | ● | | | | | | ● | | ● | | |
| Schukken et al., 2011 | ● | ● | | | | ● | 1- Untreated | | | | | | | | | | | 2 > 1 |
| | | | | | | | 2- Ceftiofur 125 mg (5 d, 5×) | ● | | | ● | | | | ● | | | |
| Schukken et al., 2013 | ● | ● | ● | ● | ● | ● | 1- Cephapirin 200 mg (1 d, 2×) | ● | | | ● | | | | | | ● | 1 = 2 (gram-positive); 2 > 1 (gram-negative) |
| | | | | | | | 2- Ceftiofur 125 mg (5 d, 5×) | ● | | | ● | | | | ● | | | |
| Sérieys et al., 2005 | ● | | ● | ● | ● | ● | 1- Penethamate hydroiodide 7.5 mg/kg of BW (3 d, 3×)[9] | | ● | | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- Cloxacillin 200 mg + ampicillin 75 mg (3 d, 3×) | ● | | | ● | | | | | ● | | |
| Shpigel et al., 1997 | ● | | | | | | 1- Cloxacillin 200 mg + ampicillin 75 mg (2 d, 3×) | ● | | | ● | | | | | ● | | 2 = 3 = 4 > 1 |
| | | | | | | | 2- Cefquinome 75 mg (2 d, 3×) | ● | | | ● | | | | ● | | | |
| | | | | | | | 3- Cefquinome 1 mg/kg of BW (2 d, 2×) | | ● | | ● | | | | ● | | | |
| | | | | | | | 4- [2] + [3] | | | ● | ● | | | | ● | | | |
| Sol et al., 2000 | | | ● | | | | 1- Cefazolen 200 mg (2 d, 3×) | ● | | | ● | | | | | | ● | 1 = 2 = 3 = 4 = 5[12] |
| | | | | | | | 2- Oxyminopenicillin 200 mg (2 d, 3×) | ● | | | ● | | | | | ● | | |
| | | | | | | | 3- Cloxacillin 200 mg + ampicillin 75 mg (2 d, 3×) | ● | | | ● | | | | | ● | | |
| | | | | | | | 4- Rifamycin 300 mg + trimethoprim 200 mg (2 d, 3×) | ● | | | ● | | | | | ● | | |
| | | | | | | | 5- Cephalotin 200 mg + colistin 200 mg (2 d, 3×) | ● | | | ● | | | | ● | | | |
| Swinkels et al., 2013 | | | ● | | | | 1- Cefquinome 75 mg (2 d, 3×) | ● | | | ● | | | | ● | | | 1 = 2 |
| | | | | | | | 2- Cefquinome 75 mg (5 d, 6×) | ● | | | ● | | | | ● | | | |
| Swinkels et al., 2014 | ● | | ● | ● | ● | ● | 1- Cefquinome 7 5mg (2 d, 3×) | ● | | | ● | | | | ● | | | 2 > 1 (Nag. strep.) |
| | | | | | | | 2- Cefquinome 75 mg (5 d, 6×) | ● | | | ● | | | | ● | | | |
| Taponen et al., 2003a | | | ● | | | | 1- [Penicillin procaine 600,000 IU (1 d, 1×)] + [Penicillin procaine 20 mg/kg of BW (4 d, 4×)] | | | ● | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- [Penicillin procaine 500,000 IU + neomycin 300 mg (1 d, 1×)] + [Penicillin procaine 20 mg/kg of BW (4 d, 4×)] | | | ● | ● | | | | | ● | | |
| Taponen et al., 2003b | | | ● | | | | 1- Penicillin procaine 20 mg/kg of BW (5 d, 5×) | | ● | | ● | | | | | ● | | 3 > 1 (β-lactamase negative); 2 = 4 (β-lactamase positive) |
| | | | | | | | 2- [Amoxycillin 200 mg + clavulanic acid 50 mg (4 d, 4×)] + [Amoxycillin 7 mg/kg of BW + clavulanic acid 1.75 mg/kg of BW (5 d, 5×)] | | | ● | | ● | | | | ● | | |
| | | | | | | | 3- [Penicillin procaine 500 mg + neomycin 300 mg (4 d, 4×)] + [Penicillin procaine 20 mg/kg of BW (5 d, 5×)] | | | ● | ● | | | | | ● | | |
| | | | | | | | 4- Spiramycin 10 mg/kg of BW (5 d, 5×) | | ● | | ● | | | | ● | | | |

*Continued*

**Table 2 (Continued).** Summary of findings from individual studies

| Reference | Pathogen[1] | | | | | | Treatment protocol (days under treatment, total number of treatments)[2] | Route[3] | | | Supportive therapy[4] | | | | Category[5] | | | Findings[6] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Escherichia coli* | *Klebsiella* spp. | *Staphylococcus aureus* | NAS | Nag. strep. | Other | | Intramammary | Systemic | Combined | None | IMM AIM | Systemic AIM | FM | HSTP (CIA) | HP (CIA) | HI | |
| Tomazi et al., 2018 | ● | ● | ● | ● | ● | ● | 1- Cephapirin 200 mg (2 d, 4×) | ● | | | | | ● | | | | ● | 1 = 2 |
| | | | | | | | 2- Tetracycline 200 mg + neomycin 250 mg + bacitracin 2 8mg (2 d, 4×) | ● | | | | ● | | | | ● | | |
| Truchetti et al., 2014 | | | ● | | | ● | 1- Ceftiofur 125 mg (2 d, 2×) | ● | | | ● | | | | ● | | | 2 > 1 |
| | | | | | | | 2- Ceftiofur 125 mg (8 d, 8×) | ● | | | ● | | | | ● | | | |
| Vasquez et al., 2016 | ● | ● | ● | ● | ● | ● | 1- Hetacillin potassium (eq. 62.5 mg of ampicillin; 3 d, 3×)[10] | ● | | | ● | | | | | ● | | 1 = 2 |
| | | | | | | | 2- Ceftiofur 125 mg (5d, 5×) | ● | | | ● | | | | ● | | | |
| Wilson et al., 1996 | ● | ● | ● | ● | ● | ● | 1- Cloxacillin 200 mg (1.5 d, 3×) | ● | | | ● | | | | | | ● | 1 = 2 |
| | | | | | | | 2- Florfenicol 750 mg (1.5 d, 3×) | ● | | | ● | | | | | | ● | |
| Wraight, 2003 | ● | | ● | | ● | ● | 1- Cefuroxime sodium 250 mg (1.5 d, 3×) | ● | | | ● | | | | | | ● | 1 = 2 |
| | | | | | | | 2- Cloxacillin 300 mg (6 d, 3×) | ● | | | ● | | | | | | ● | |

[1]Pathogen causing mastitis from which individualized cure rates are available. NAS = non-*aureus* staphylococci; Nag. strep. = non-*agalactiae* streptococci; Other = other bacteria including grouped results (e.g., coliforms, gram-positive, *Staphylococcus* spp.).

[2]For combined therapies, antimicrobial or group of antimicrobials administered using each route are separated by brackets as follows: [intramammary] + [systemic]. SID = once a day (*semel in die*).

[3]Route of administration of the antimicrobial(s).

[4]IMM AIM = intramammary anti-inflammatories; systemic AIM = systemic anti-inflammatories; FM = frequent milking.

[5]World Health Organization Antimicrobial Categorization based on the fifth revision of Critically Important Antimicrobials for Human Medicine. HSTP (CIA) = highest-priority critically important antimicrobials; HP (CIA) = high-priority critically important antimicrobials; HI = highly important antimicrobials.

[6]Reported findings in terms of bacteriological cure rates.

[7]Protocol as follows: intramuscular oxytocin at a dose of 80 IU at first milking and 20 IU at each of the 5 subsequent milkings.

[8]Protocol as follows: subcutaneous penethamate hydriodide at a dose of 10 g on d 0, followed by 5 g on d 1.

[9]Protocol as follows: intramuscular penethamate hydriodide at a dose of 15 mg/kg of BW on d 0, followed by 7.5 mg/kg of BW on d 2 and 3.

[10]Quantity of hetacillin potassium equivalent to 62.5 mg of ampicillin as per product label.

[11]Study carried out in multiple phases.

[12]Results from extended therapy were not considered.

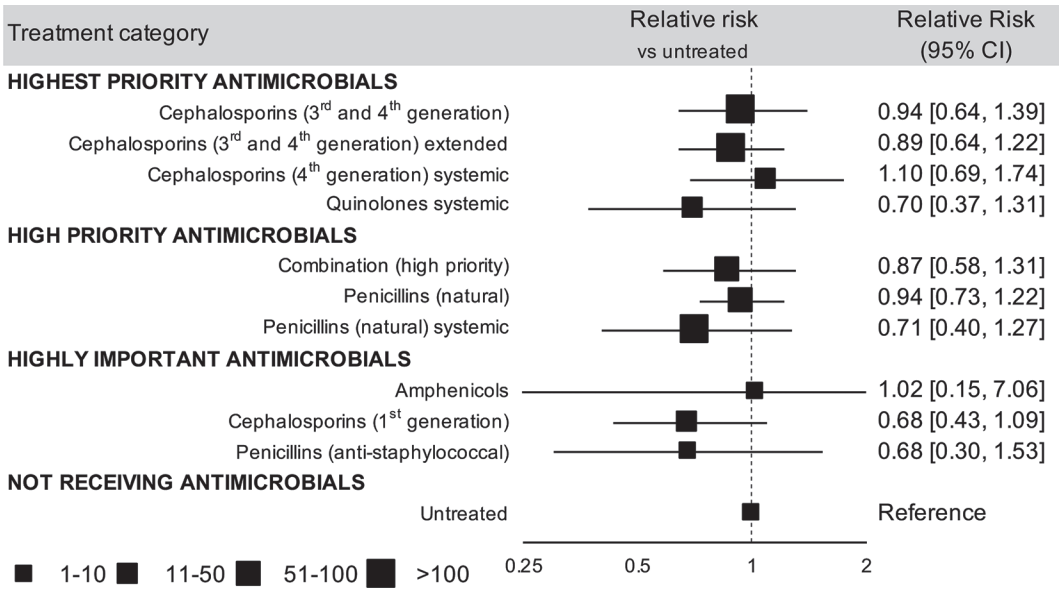


**Figure 2.** Forest plot of relative risks (and respective 95% CI) of bacteriological cure of nonsevere bovine clinical mastitis caused by *Escherichia coli*. Untreated cows were considered as the reference group. Marker size is related to number of units (cows or quarters) enrolled per treatment category after the World Health Organization categorization of medically important antimicrobials.

### *Non-Aureus Staphylococci*

Fifteen studies reported on bacteriological cure rates of antimicrobials used to treat nonsevere CM caused by non-*aureus* staphylococci. From this total, 13 were eligible for inclusion in networks, wherein relatively high heterogeneity values were detected in comparison to models for remaining bacterial species (Supplemental Table S5, https://doi.org/10.3168/jds.2020-18365). For treatment of nonsevere CM caused by non-*aureus* staphylococci, no protocol with use of CIA resulted in increased bacteriological cure when compared with protocols using non-CIA (Figure 4; Supplemental Table S6, https://doi.org/10.3168/jds.2020-18365). Protocols with intramammary administration of third or fourth generation cephalosporins, macrolides or combinations containing CIA of highest priority, were comparable to protocols with intramammary administration of first or second generation cephalosporins for promoting bacteriological cure of nonsevere CM caused by non-*aureus* staphylococci (Figure 4). No eligible study reported bacteriological cure rates for untreated cows with nonsevere CM caused by non-*aureus* staphylococci.

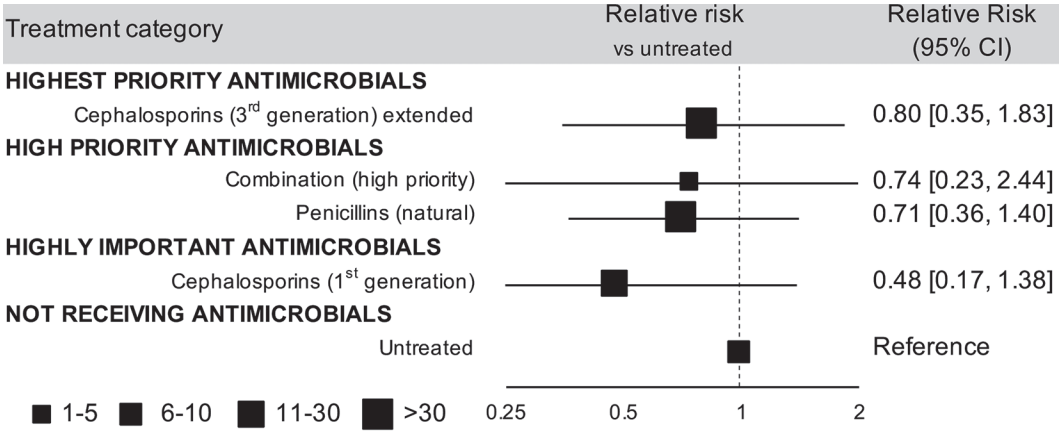


**Figure 3.** Forest plot of relative risks (and respective 95% CI) of bacteriological cure of nonsevere bovine clinical mastitis caused by *Klebsiella* spp. Untreated cows were considered as the reference group. Marker size is related to number of units (cows or quarters) enrolled per treatment category after the World Health Organization categorization of medically important antimicrobials.

### Staphylococcus Aureus

From the 22 studies reporting on antimicrobial bacteriological cure rates for treatment of nonsevere CM caused by *S. aureus*, 17 were included in our final *S. aureus* network. Sensitivity analysis indicated that *S. aureus* results depended on inclusion of a study classified as having high risk of bias (Aguilera, 1983), which was therefore excluded. Additionally, our selected *S. aureus* model did not include 4 studies with use of protocols based on intramammary administration of anti-inflammatories due to a marked increase in heterogeneity (Supplemental Table S7, https://doi.org/10.3168/jds.2020-18365). The bacteriological cure rate of nonsevere CM caused by *S. aureus* was not different when comparing CIA versus non-CIA; consequently, no CIA category was superior to all non-CIA categories (Supplemental Table S8, https://doi.org/10.3168/jds.2020-18365; Figure 5). No study reporting on spontaneuous bacteriological cure rates of nonsevere CM caused by *S. aureus* could be included in our networks.

### Non-Agalactiae Streptococci

Twenty-one studies reported on bacteriological cure rates of antimicrobials for treating nonsevere CM caused by non-*agalactiae* streptococci, from which 20 were eligible for inclusion in networks. No study or protocol was excluded from networks according to our sensitivity analysis (Supplemental Table S9, https://doi.org/10.3168/jds.2020-18365). No protocol using CIA resulted in higher bacteriological cure rates of nonsevere CM caused by non-*agalactiae* streptococci compared with protocols using non-CIA (Supplemental Table S10, https://doi.org/10.3168/jds.2020-18365). Hence, there was no evidence to support need of CIA for treating nonsevere CM caused by non-*agalactiae* streptococci (Supplemental Table S10; Figure 6). Nevertheless, administration of antimicrobials was associated with higher probability of bacteriological cure.

### Bias Assessments and Network Structures

Nearly half of the studies used protocols for treatment allocation precluding adequate allocation concealment (Table 3). For most studies, distribution of potential confounders pre-intervention was similar between groups. A potential source of bias common to all studies was housing of cows; it was unclear whether cows from different intervention groups were housed at the same facilities. In general, implementation of blinding at either the performance- or detection-level was infrequent. The most common reason for nonblinded designs was use of very distinct treatment protocols (e.g., 2 vs. 8 d of treatment) in a study. A single study was flagged out as a potential source of significant bias (Table 3) and had its effect on networks evaluated using sensitivity analysis. Additionally, we did not detect evidence of reporting bias across studies (Supplemental Figures S1–S5, https://doi.org/10.3168/jds.2020-18365). Finally, for all pathogens, network structures revealed a high degree of indirectness, low density, and limited number of direct comparisons of CIA versus non-CIA (Supplemental Figures S6–S10, https://doi.org/10.3168/jds.2020-18365).

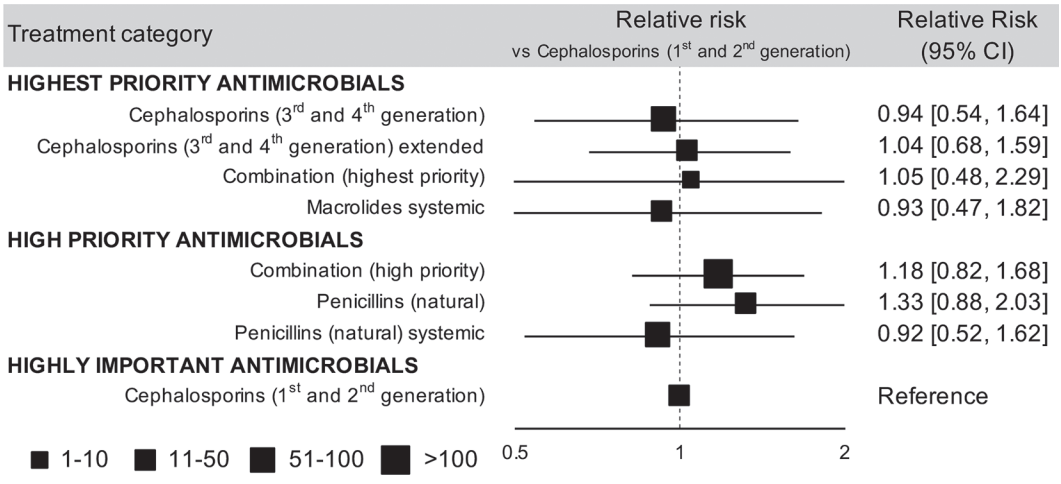


**Figure 4.** Forest plot of relative risks (and respective 95% CI) of bacteriological cure of nonsevere bovine clinical mastitis caused by non-*aureus* staphylococci. Cows receiving intramammary administration of first- or second-generation cephalosporins were considered as the reference group. Marker size is related to number of units (cows or quarters) enrolled per treatment category after the World Health Organization categorization of medically important antimicrobials.

### Qualitative Results

Two of the 4 studies that could not be included in the network meta-analyses reported on bacteriological cure rates of nonsevere coliform CM (Lavy et al., 1995; Schukken et al., 2011). A positive effect of CIA to treat nonsevere *E. coli* CM was reported from one trial (Schukken et al., 2011), in contrast with other sources of evidence (Guterbock et al., 1993; Roberson, 1998; Roberson et al., 2004). In addition, no difference between protocols based on increasing dosages of florfenicol (a non-CIA) for treating CM caused by *E. coli* was reported by the second trial (Lavy et al., 1995). In the 2 remaining trials, gram-positive bacteria were responsible for the majority of CM. Systemic therapy (intra-arterial) with use of high-priority CIA was more effective in treating CM caused by either *Streptococcus agalactiae* or *S. aureus* than intramammary administration of the same antimicrobials (Aguilera, 1983). Additionally, 2 antimicrobial formulations, both containing high-priority CIA, were equally effective to treat cows with CM infected mostly by *S. aureus*, non-*aureus* staphylococci, and non-*agalactiae* streptococci (McDougall, 2003).

## DISCUSSION

To our knowledge, this is the first systematic review comparing efficacy of CIA and non-CIA to treat nonsevere CM caused by the most commonly isolated bovine mastitis pathogens worldwide. Findings from this study are important to inform public strategies aimed to promote antimicrobial stewardship in veterinary medicine.

There is an urgent need to restrict and control use of antimicrobials in livestock due to potential adverse effects on human health (Tang et al., 2017). Veterinarians and farmers should choose antimicrobial agents based on a combination of factors including efficacy for treating specific pathogens and potential emergence of antimicrobial resistance following therapy. Although prevalence of antimicrobial resistance in dairy herds has not increased in the last 4 decades (Oliver and Murinda, 2012), use of CIA in food-producing animals has come under scrutiny in recent years (Apostolakos and Piccirillo, 2018) because of potential negative effects on human and environmental health. Veterinarians should choose non-CIA of comparable or superior efficacy for treating infections in animals as part of any antimicrobial stewardship program. We concluded that CIA and non-CIA have comparable efficacy to treat nonsevere CM in dairy cattle caused by the most commonly isolated pathogens that cause mastitis worldwide. Hence, assuming all other variables that affect safety, choice, and use of antimicrobials are held equal, no adverse effects in terms of animal health and welfare should be expected by ceasing use of CIA for treating nonsevere CM in herds where no clinical need for CIA had been established (Turner et al., 2018).

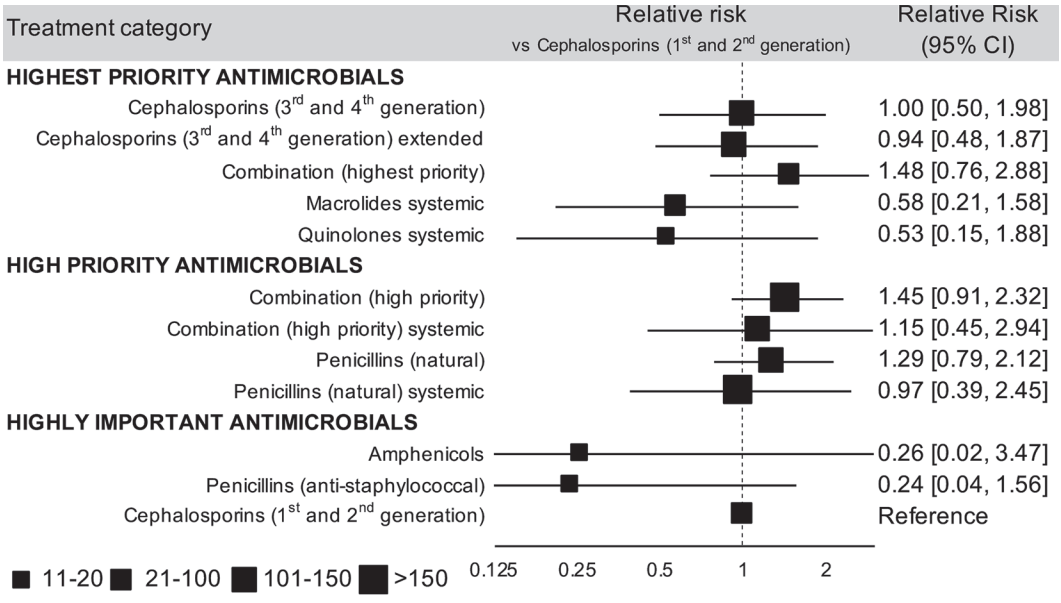


**Figure 5.** Forest plot of relative risks (and respective 95% CI) of bacteriological cure of nonsevere bovine clinical mastitis caused by *Staphylococcus aureus*. Cows receiving intramammary administration of first- or second-generation cephalosporins were considered as the reference group. Marker size is related to number of units (cows or quarters) enrolled per treatment category after the World Health Organization categorization of medically important antimicrobials.

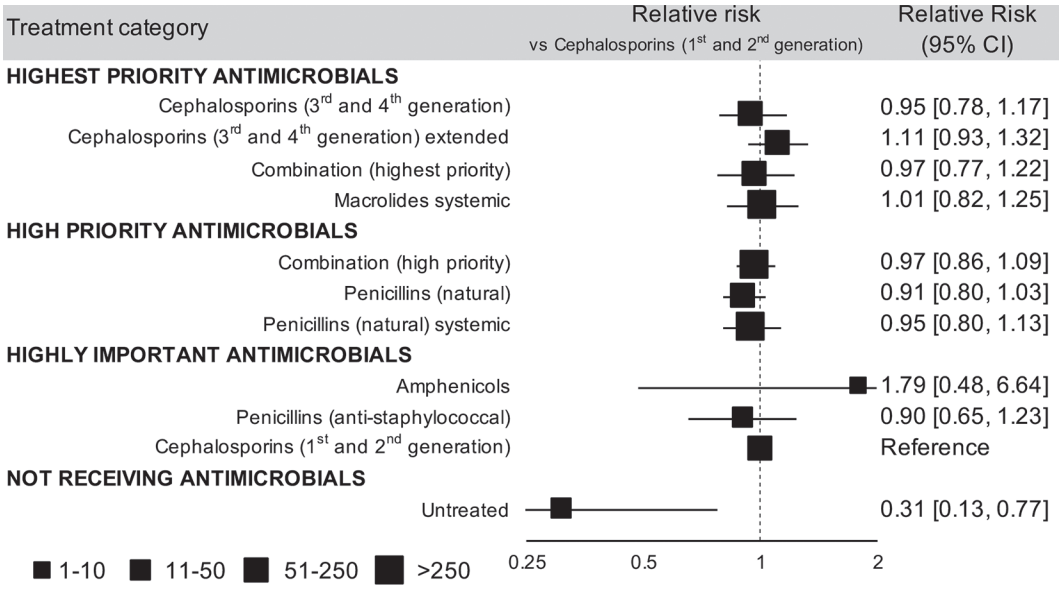


**Figure 6.** Forest plot of relative risks (and respective 95% CI) of bacteriological cure of nonsevere bovine clinical mastitis caused by non-*agalactiae* streptococci. Cows receiving intramammary administration of first- or second-generation cephalosporins were considered as the reference group. Marker size is related to number of units (cows or quarters) enrolled per treatment category after the World Health Organization categorization of medically important antimicrobials.

Spontaneous cure of intramammary infections and CM caused by gram-positive bacteria is unlikely (Hillerton and Kliem, 2002; Roy et al., 2009); however, the same is not true for CM caused by gram-negative bacteria (Fairbrother et al., 2015). Our findings suggest that antimicrobial treatment is not necessary for nonsevere cases of CM caused by *E. coli* and *Klebsiella* spp. Whereas our findings were in agreement with previous studies on bacteriological cure rates of nonsevere CM caused by *E. coli* (Roberson et al., 2004; Ruegg, 2018; Fuenzalida and Ruegg, 2019), recent evidence demonstrates that antimicrobial therapy of nonsevere CM caused by *Klebsiella pneumoniae* is associated with higher bacteriological cure rates of treated cows (Fuenzalida and Ruegg, 2019). Unfortunately, included studies reporting bacteriological cure rates for nonsevere CM caused by *Klebsiella* spp. had a relatively low sample size, limiting our power to assess the need of antimicrobials for treating CM caused by this important pathogen. Nevertheless, irrespective of bacteriological cure, most cows with nonsevere CM caused by coliforms are clinically cured within 1 wk of onset of CM, regardless of treatment. In addition, there are no differences in terms of mastitis recurrence, apparent culling rates, and voluntary dry-off between treated and nontreated quarters affected by coliforms (Fuenzalida and Ruegg, 2019), suggesting that empirical antimicrobial treatment of nonsevere CM caused by coliforms using CIA is irresponsible. In that regard, use of culture-based, selective treatment programs where antimicrobials are not used for cases of mastitis caused by gram-negative bacteria can be a valuable alternative. Nonetheless, although our findings supported use of selective treatment of CM, we must emphasize that our main objective was to contrast CIA versus non-CIA for treatment of nonsevere CM.

The WHO list of CIA was developed with regard to the importance of antimicrobials in human medicine; cost was not a primary factor for classifying an antimicrobial as critically important. This classification scheme informs decision-making, particularly strategies for antimicrobial administration in food-producing animals (Collignon et al., 2016). In veterinary epidemiology, decision-making should factor in expected economical outcomes (James, 2005); costs of interventions are expected to directly affect dynamics of animal diseases. Restrictions of antimicrobial use in dairy cattle could increase milk price, decreasing gross revenue for dairy farmers as well as affecting markets (Lhermie et al., 2018). A framework for tackling antimicrobial resistance not only relies on reducing overall antimicrobial consumption, but also on ensuring that the most effective narrow-spectrum antimicrobial is used when necessary (Om et al., 2016). Our findings support policies to reduce or eliminate use of CIA in dairy herds. Yet, despite a palpable sense of urgency due to alarming antimicrobial resistance rates worldwide, we must stress that phasing out use of CIA to treat CM will depend on

**Table 3.** Risk of bias as assessed using Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) guidelines from experimental full-text manuscripts included[1]

| | Selection bias | | | Performance bias | | Detection bias | | Attrition bias | Reporting bias |
|---|---|---|---|---|---|---|---|---|---|
| Reference | Allocation concealment | Sequence generation | Baseline characteristics | Random housing | Blinding | Random assessment | Blinding | Incomplete data | Selective reporting |
| Aguilera, 1983 | | | | Unclear | | √ | | | |
| Bradley and Green, 2009 | | √ | | Unclear | √ | √ | √ | √ | √ |
| Bryan et al., 2016 | √ | √ | √ | Unclear | √ | √ | √ | √ | √ |
| Cortinhas et al., 2016 | | √ | √ | Unclear | | √ | | √ | √ |
| Guterbock et al., 1993 | √ | √ | √ | Unclear | | √ | | √ | √ |
| Hillerton and Kliem, 2002 | | √ | √ | Unclear | | √ | | √ | √ |
| Kalmus et al., 2014 | | √ | √ | Unclear | | √ | | √ | √ |
| Lavy et al., 1995 | √ | √ | √ | Unclear | | √ | | √ | √ |
| McDougall, 1998 | √ | √ | √ | Unclear | | √ | | √ | √ |
| McDougall, 2003 | √ | √ | √ | Unclear | √ | √ | √ | √ | √ |
| McDougall et al., 2007a | √ | √ | √ | Unclear | √ | √ | √ | √ | √ |
| McDougall et al., 2007b | | √ | √ | Unclear | √ | √ | √ | √ | √ |
| Oliver et al., 2004 | | √ | √ | Unclear | | √ | | √ | √ |
| Pyorala and Pyorala, 1998 | | √ | √ | Unclear | | √ | | √ | √ |
| Roberson, 1998 | √ | √ | √ | Unclear | | √ | | √ | √ |
| Roberson et al., 2004 | √ | √ | √ | Unclear | | √ | | √ | √ |
| Schukken et al., 2011 | | √ | √ | Unclear | √ | √ | √ | √ | √ |
| Schukken et al., 2013 | | √ | √ | Unclear | | √ | | √ | √ |
| Sérieys et al., 2005 | | √ | √ | Unclear | | √ | | √ | √ |
| Shpigel et al., 1997 | | √ | √ | Unclear | √ | √ | √ | √ | √ |
| Sol et al., 2000 | | √ | √ | Unclear | | √ | √ | √ | √ |
| Swinkels et al., 2013 | √ | √ | √ | Unclear | | √ | | √ | √ |
| Swinkels et al., 2014 | √ | √ | √ | Unclear | | √ | | √ | √ |
| Taponen et al., 2003a | | √ | √ | Unclear | √ | √ | √ | | √ |
| Taponen et al., 2003b | | √ | √ | Unclear | | √ | | √ | √ |

[1]A check mark indicates that the item was thoroughly described, minimizing risk of bias.

other factors, for example, whether alternatives demonstrate efficacy in cases of intramammary infections caused by bacteria resistant to non-CIA. Additionally, despite comparable efficacy, economic factors such as costs of specific antimicrobials, duration of milk withdrawal, duration of therapy and route of administration must also be included in the equation when selecting an antimicrobial for mastitis treatment. Therefore, our findings should be used as an important part of a discussion for implementing strategies to manage CM in dairy herds that align with international recommendations for combating antimicrobial resistance.

There is not enough evidence to support exclusive use of supportive therapy (e.g., frequent milking and homeopathy) for treating nonsevere CM in dairy cattle (Morin et al., 1998; Francoz et al., 2017). Use of supportive therapy might even be detrimental to udder health (Roberson et al., 2004). We handled use of supportive therapy in 3 ways: (1) by performing a set of sensitivity analysis, (2) by excluding studies where treatment protocols differed exclusively on use of supportive therapy, and (3) by extracting data from groups not receiving supportive therapy in studies reporting individualized cure rates for $\geq 2$ antimicrobials, with and without supportive therapy. For all pathogens except for *S. aureus*, findings and model estimates were comparable, despite inclusion of protocols with use of supportive therapy. Surprisingly, for *S. aureus,* exclusion of protocols with use of intramammary anti-inflammatories resulted in a sharp decrease in heterogeneity estimates, suggesting that efficacy of a subset of antimicrobials depended on intramammary administration of anti-inflammatories. It was suggested that bacterins administered in conjunction with pirlimycin might increase probability of bacteriological cure for cows with intramammary infections caused by *S. aureus* (Luby and Middleton, 2005), potentially due to reduced inflammation resulting from a focused immune response. Similarly, reduced inflammation due to intramammary administration of anti-inflammatories could potentially affect the overall probability of cure, resulting in increased heterogeneity estimates as detected in our networks.

The goal of antimicrobial therapy is to enhance bacterial clearance; efficacy of products used to treat CM is initially evaluated using estimates of bacteriologic cure rates (Ruegg, 2018). We excluded studies that did not report bacteriological cure rates; our choice of outcome relied on a balance between number of eligible studies and heterogeneity. Ideally, one would use an elaborate metric of cure based on lack of clinical signs and elimination of causative pathogen (e.g., complete cure). Yet, adoption of such a metric would inevitably lead to exclusion of a majority of studies, as this information was not available. Alternatively, a large increase in number of eligible studies can be achieved by inclusion of studies reporting clinical cure. Nonetheless, as mastitis cure rates depend on the causal pathogens (Vasquez et al., 2016), the information provided by clinical cure in absence of bacteriological culturing is potentially a source of heterogeneity when evaluating effects of antimicrobials. If, for instance, treatment assignment depended on underlying causal bacteria in studies reporting exclusively on clinical cure, there would have been increased heterogeneity or inconsistency in our networks. Such discrepancy could arise, for example, from distinct pathogen-specific incidence rates that are commonly reported from countries (Oliveira et al., 2013; 2015).

Some limitations of this study must be acknowledged. First, non-*agalactiae* streptococci and non-*aureus* staphylococci are diverse groups. Antimicrobial efficacy depends on the causal pathogen, and it is unclear whether efficacy of a specific antimicrobial will be similar for CM cases caused by different pathogens (e.g., *S. uberis* versus *S. dysgalactiae* or *S. chromogenes* versus *S. epidermidis*). Similarly, in the absence of molecular identification techniques such as MALDI-TOF, non-*aureus* staphylococci were typically only identified at the group level when based on culture alone. Without differentiating non-*aureus* staphylococci species, it is difficult to distinguish between a failure to cure and a successful cure followed by a new IMI by another non-*aureus* staphylococci species. Additionally, network meta-analyses inevitably depend on some degree of grouping. Although we tested 2 antimicrobial categorization schemes, a wide variety of antimicrobial treatment protocols receive the same WHO categorization. Nevertheless, the relatively low heterogeneity and nonsignificant inconsistency estimates were reassuring; not only was the between-study variance for same comparison in a species relatively low, but direct and indirect sources of evidence were in overall agreement. Second, despite nonsignificant inconsistency estimates, the majority of evidence from networks was indirect and should be interpreted accordingly. Of note, the limited number of studies and elevated number of treatment protocols resulted in low-density networks, which was expected due to diversity of treatment protocols for CM available worldwide. Additionally, low heterogeneity detected in the networks was mostly a consequence of lack of common treatment arms across studies. In this scenario, the assumption of transitivity (no systematic differences among comparisons of same treatments) becomes crucial (Salanti et al., 2014). Third, for CM cases caused by *Klebsiella* spp., and for specific antimicrobial categories (e.g., amphenicols, anti-staphylococcal penicillins), low sample size reduced our probability of detecting meaningful associations; absence of statistical significance at 5% level in this

case could have been due to lack of statistical power. Moreover, our study ignored the concept of margin of noninferiority. For bacteriological cure rates differing within a pre-established value, its adoption would ensure that antimicrobials from separate categories were deemed noninferior to each other in terms of overall efficacy, despite presence of statistical significance. Finally, findings were specific for the current scenario in terms of antimicrobial alternatives for treating CM; development of new antimicrobials, formulations, or treatment protocols was not captured by our models. Similarly, CIA and non-CIA included in our analysis did not encompass all possible antimicrobial molecules and classes from these categories. Therefore, we do not recommend extrapolation of our findings to noninincluded antimicrobials or treatment protocols.

## CONCLUSIONS

Our findings support that antimicrobial treatment does not improve the outcome for nonsevere CM caused by *E. coli*. Additionally, CIA and non-CIA had comparable efficacy for treating nonsevere CM caused by the 5 most prevalent mastitis-causing pathogens or group of pathogens worldwide (*S. aureus*, non-*aureus* staphylococci, non-*agalactiae* streptococci, *E. coli*, and *Klebsiella* spp.). Findings from this study support public strategies that promote responsible antimicrobial stewardship in veterinary medicine.

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