***Title***

Literature review: Antimicrobial susceptibility of bovine staphylococcal mastitis isolates on organic vs. conventional dairy farms

C. E. Jeffrey1 and J. W. Barlow1

1 Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT 05405

Corresponding author: Caitlin Jeffrey

Department of Animal and Veterinary Sciences,

202 Terrill Building,

University of Vermont,

Burlington, VT 05405

Phone: 802-656-1395

Email: [caitlin.jeffrey@uvm.edu](mailto:caitlin.jeffrey@uvm.edu)

***Abstract***

An unfortunate consequence of any antimicrobial use is the potential to select for the emergence of resistant strains of bacteria in a population. A unique opportunity in which to assess the effect of antimicrobial use on resistance of mastitis pathogens is to compare dairy farms which are managed “conventionally” to those that are managed “organically.” Without the selective pressure of antimicrobial usage (as on organic dairies), it would be expected that resistant bacterial strains would gradually be replaced by susceptible strains if an advantage was no longer conferred by carriage of antimicrobial resistance (AMR) genes. The objective of this narrative review is to summarize studies which compared the relationship between antimicrobial usage at the farm level (organic vs. conventional) and AMR of bovine staphylococcal mastitis isolates, the predominant group of bacteria causing intramammary infections in dairy cattle globally. Other potential explanatory factors for differing antimicrobial susceptibility of staphylococci causing intramammary infections are described. These include differences in AMR carriage between staphylococcal species and various risk factors associated with the prevalence of different species causing intramammary infections in a particular herd. Overall, studies comparing AMR of mastitis-associated staphylococci between herds under organic management and herds managed conventionally find either no difference or that isolates originating from organic farms exhibit slightly more susceptibility. Although some level of resistance was observed against a number of antimicrobials important for veterinary medicine (cephalosporins, penicillin, tetracycline), overall resistance of mastitis-associated staphylococci is generally low and the most commonly-used mastitis treatments are still effective. Studies exploring this issue varied widely in their approach, including use of differing methodology to determine susceptibility patterns and variation in sampling scheme. Most studies were carried out in either the US or Europe. This is somewhat problematic, as definitions of “organic” differ for dairies in the EU (where antimicrobial usage is still allowed, but is more tightly regulated and limited) and the US (any animal treated with antimicrobials must leave the herd). However, the overall conclusions from studies comparing the two different management systems are still informative. Directions for future work could include comparing AMR for staphylococci between these two systems while controlling for species, comparison of predominant strain types within a given species between organic and conventional farms, or long-term studies of farms transitioning from conventional to organic status to better understand what types of AMR are maintained in organic dairy herds and for how long.

***Introduction***

Effective antimicrobial therapy is a cornerstone of livestock veterinary medicine, maintaining the health of animals producing food and fiber to support the global population and alleviating suffering due to infectious disease. However, use of antimicrobial agents is inherently a “powerful selective force that promotes the emergence of resistant strains,” and the cumulative effect of antibiotic use in general has “clearly been to increase the prevalence of resistance in the population [of bacteria] as a whole” (Lipsitch and Samore, 2002). Resistance to antimicrobials can be acquired by bacteria in multiple ways. Spontaneously occurring genetic mutations (passed vertically to daughter cells) can confer antimicrobial resistance, but more commonly it is acquired by the horizontal transfer of mobile DNA elements from a donor cell, often another species of bacteria (Chambers, 2001; Sefton, 2002). In the case of horizontal transfer, antimicrobial resistance genes can become rapidly and widely disseminated throughout a bacterial population. This occurs either by further genetic exchanges between the newly-resistant strain and susceptible strains, or by clonal spread of the newly-resistant strain itself (Chambers, 2001).Although the interplay between development of resistance and antimicrobial use is complex and multifactorial, it is generally accepted that antimicrobial resistance (AMR) is potentially amplified in both human healthcare environments and on farms, where frequent exposure to antimicrobial compounds can select for resistant populations of bacteria (Parker et al., 2024). A direct temporal relationship between antimicrobial use and resistance has been described, both in human healthcare settings over the long-term (López-Lozano et al., 2000) and in transient increases in resistant fecal bacteria in cattle (Stabler et al., 1982; Langford et al., 2003; Berge et al., 2005; Lowrance et al., 2007). It has been suggested that antimicrobial usage in food animals could negatively affect human health by influencing the selection of drug-resistant foodborne pathogens (Yan and Gilbert, 2004). However, the risk of transmission of resistant bacteria between farm systems and humans is not fully understood; selection for resistant bacteria and transfer of AMR genes occurs through a variety of mechanisms, and is not always linked to use of a specific antibiotic (Mathew et al., 2007).

The most “obvious selection pressure for AMR” on cattle farms is the use of antimicrobials for treating sick animals (Call et al., 2008). Specifically, this can promote AMR on cattle farms by two potential mechanisms: 1) treatment with antimicrobials provides a competitive advantage for strains that carry resistance to that particular drug, allowing the relative proportion of resistant bacteria in a populations to increase; and 2) if resistance genes are harbored on horizontally transmissible elements (plasmids or conjugative transposons), strains carrying these elements can then successfully disseminate them to new, previously-susceptible bacteria (Call et al., 2008). The primary reason for antimicrobial drug usage in adult dairy cows in the US is for treatment of mastitis (Pol and Ruegg, 2007b). Bacteria belonging to the genus *Staphylococcus*, which broadly includes the major mastitis pathogen *Staphylococcus aureus* and a heterogeneous group of bacteria known as the non-aureus staphylococci and mammaliicocci (NASM), are the predominant pathogens causing intramammary infections (IMI) in dairy animals worldwide (as summarized in De Buck et al., 2021). A limited number of antimicrobials are approved for treatment of mastitis in lactating dairy cattle in the US, including various β-lactams (penicillin, cephapirin, ceftiofur, amoxicillin, hetacillin, and cloxacillin) and one lincosamide (pirlimycin) (FARM, 2020). At this time, *S. aureus,* NASM, and other mastitis pathogens are generally susceptible to the antibiotics currently used to treat IMI in the US (Kolar et al., 2024; Pol and Ruegg, 2007b). NNotably some studies from other regions report *S. aureus* and NASM exhibiting moderate resistance against penicillin(add references here). However, efforts to continue surveying and understanding the AMR patterns for these ubiquitous mastitis pathogens is warranted. The importance of *S. aureus* as a human pathogen is well-established (Tong et al., 2015), and virulence genes known to cause disease in both humans and animals have been demonstrated in NASM isolates from bovine IMI (Park et al., 2011; Unal and Cinar, 2012, Naushad et al., 2018). Additionally, transmission of resistance genes between different staphylococcal species have led to the idea that NASM may act as a “reservoir” of AMR for more pathogenic staphylococcal species such as *S. aureus* (Cuny et al., 2017; Feßler et al., 2018; Khazandi et al., 2018).

A unique opportunity in which to assess the effect of antimicrobial use on AMR of these important mastitis pathogens is to compare dairy farm systems which are managed “conventionally” to those that are managed “organically.” Although the definition can differ by region (namely, the US and EU; see below), antimicrobial usage on “organic” dairies is usually less or non-existent when compared to “conventional’” dairy farms. In what appears to be a review article focused on the US dairy industry, Philips and Heins noted the definition of conventional farms is vague. They defined conventional dairy farms “non-organic livestock systems that keep animals in total indoor confinement and have the ability to utilize treatments that are not allowed in organic practices, such as antibiotics, when necessary.” They appear to recognize that in the US there is a population of herds that graze cattle but are not certified organic. Might note that US organic certification is a marketing status not a food safety or quality regulatory framework, although US organic herds must follow the Pasteurized Milk Ordinance (PMO). US Organic certification is about using what is allowable and most synthetic products including commercially available antimicrobials approved for prevention or treatment of mastitis are not on the allowed substances list. *This is getting a little long*  When comparing bacterial isolates of bovine origin from these two types of systems, the general hypothesis is that AMR would be expected to diminish in prevalence when antimicrobial use is decreased or discontinued. Without the selective pressure of antimicrobial usage (as on organic dairies), bacterial strains containing resistance genes would gradually be replaced by susceptible strains, as selective advantage is no longer conferred by AMR carriage (assuming AMR carriage incurs a fitness cost; see below). The goal of this narrative review is to summarize studies which compared the relationship between antimicrobial usage at the farm level (organic vs. conventional) and antimicrobial susceptibility of bovine staphylococcal mastitis isolates.

***Limitations and caveats for comparisons between studies***

An important qualification when considering the body of work comparing resistance patterns of mastitis pathogens between management systems is that “organic” dairies differ between the US and Europe, where the majority of these studies have been carried out. Organic regulations in European countries still allow for some antimicrobial use (albeit with extended withdrawal periods and stricter veterinary oversight; EU Commission, 2024), while organic regulations in the US mandate that any animal treated with antimicrobials be permanently removed from the herd (USDA, 2024). The level of on-farm antimicrobial usage (and therefore selective pressure for resistance) therefore differs between European and US dairies, making comparisons between studies carried out under these varying regulations somewhat complicated. Specific rules for both organic dairy production certifications have evolved over time (Dimitri and Nehring, 2022; Grodkowski et al., 2023), further adding to the nuance of what is meant by “organic” dairy production in a retrospective analysis. The specific antimicrobials approved for usage in livestock varies by country, as well as which compounds are most commonly-used (e.g., for mastitis: penicillin in Finland, Taponen 2023; cephalosporins in the US, de Campos 2021). Even within the US, the amount and type of antimicrobials used in dairy cows changes over time as new products are developed or regulations around usage shift or commercial products are removed from the market (USDA, 2009; pirlimycin removal reference). Consequently, geographic and temporal differences can affect the type and amount of antimicrobial selective pressure experienced by mastitis pathogens on dairy farms.

Direct comparison of antimicrobial sensitivity results across studies can be problematic for a number of reasons. Importantly, the methodology used to determine the minimum inhibitory concentration (MIC) or categorization of an isolate as susceptible or resistant varies between studies. *I think maybe before getting into use of genotypic analysis to predict phenotype, maybe need a few sentences on how we (CLSI) currently use phenotypic information to inform breakpoint determinations, except for methicillin (MRSA and MRSS) where presence of mecA or production of PBP 2a should be reported as methicillin/oxacillin/cefoxitin resistant, and in vitro breakpoints are not available for all antimicrobials commercially available for treatment of mastitis*. Further, inconsistencies exist between phenotypic and genotypic resistance results, due either to 1) detection of phenotypic resistance in the absence of expected genotypic determinants, or 2) phenotypic susceptibility despite the presence of genotypic determinants. For isolates of *S. aureus* associated with bovine mastitis, both of these types of discrepancies have been reported for penicillin resistance (Sampimon, 2009; Taponen et al., 2023). This also holds true for the other staphylococci; as summarized by Sampimon (2009), “agreement between phenotypic and genotypic test results for assessment of resistance of CNS of bovine origin to penicillin, oxacillin, and ML [macrolide] antibiotics depended on the antimicrobial compound of interest and on methods used to analyse and interpret test results, but was rarely perfect.” In a study by Taponen et al. (2023) comparing methods of testing for β-lactamase mediated resistance, overall agreement between phenotypic and genotypic resistance tests was moderate to substantial for staphylococci from bovine IMI. However, some inconsistencies were found between phenotypic susceptibility by disk diffusion method, the nitrocefin test to assess β-lactamase production, and PCR to detect the presence of the *blaZ, mecA*, and *mecC* genes encoding the β-lactamase gene. Disagreements have also been described within different methods of phenotypic determination of resistance for mastitis pathogens. A study comparing commercially-available broth microdilution plates (Sensititre Custom Plates) and agar disk diffusion for determining antimicrobial susceptibility of bovine IMI isolates found fair agreement overall (80.7%) between the two methods, but this varied based on the particular bacterial-antimicrobial combination tested (Palladini et al., 2023). No NASM species were included, but there was satisfactory agreement (89 to 100%) for *S. aureus* and all antimicrobial agents tested. In a study comparing Sensititre (broth microdilution) and disk diffusion for determining AMR in clinical mastitis pathogens, agreement was good for most isolate-antimicrobial MIC combinations (Saini et al., 2011). An important exception to this was that diagnostic accuracy was low when *S. aureus* was tested against both ceftiofur and oxacillin using either method. Low correlation was also found when *S. aureus* was tested against erythromycin and neomycin in another study comparing 2 dilution methods to determine MIC and disk diffusion diameters for mastitis-associated isolates (Klement et al., 2005). Further complicating comparison of AMR profiles between studies is shifting criteria for classifying an isolate as susceptible or resistant. Breakpoints for antimicrobial susceptibility testing are updated every few years, and multiple conflicting standards exist for categorization of resistant or susceptible bacteria which are dependent on geographical location (Clinical & Laboratory Standards Institute, CLSI; European Committee on Antimicrobial Susceptibility Testing, EUCAST).

Difference in sampling scheme for studies collecting milk from individual cows will affect observed prevalence of resistance in bacteria isolated from samples. Within the studies summarized in this review, sampling strategies for quartermilk and criteria for cow inclusion vary widely. Some studies included sampled cows in a herd at random or without using any specific criteria (Tikofsky et al., 2003; Bombyk et al., 2008; Garmo et al., 2010), while others used the California Mastitis test (CMT) to selectively sample cows with evidence of extant mastitis (Busato et al., 2000; Roesch et al., 2006). Bennedsgard et al. (2006) used a specific set of criteria in order to maximize their chances of sampling cows with *S. aureus* IMI specifically, while others sampled only multiparous cows in the herd (Pol and Ruegg, 2007a; McDougall et al., 2021). Sampling multiparous cows exclusively increases the likelihood samples collected will have an IMI, as increasing parity is a risk factor for mastitis generally (Barkema et al., 1998; Busato et al., 2000) and IMI with *S. aureus* specifically (Zadoks et al., 2001; Tenhagen et al., 2006). The likelihood of different NASM species causing IMI varies by parity, and resistance patterns are species-specific for NASM (see below). Therefore, sampling multiparous cows exclusively will bias which species are included and thereby the resistance profiles of mastitis pathogens described. A further consideration is whether the bacteria included were associated with cases of subclinical mastitis, clinical mastitis, or both. AMR has been shown to be more prevalent in NASM isolates associated with clinical vs. subclinical mastitis, so inclusion criteria around sample type will affect the observed AMR prevalence. Oxacillin resistance was more frequent in clinical mastitis isolates (56.5%) vs. subclinical mastitis isolates (43.9%; Frey et al., 2013), β-lactamase production was more common in subclinical vs. clinical cases (Persson Waller et al., 2011), and Wuytack et al. (2020) found carriage of the resistance gene *mecA* was proportionately higher in NASM isolates causing clinical vs. subclinical infection. However, as certain NASM are more likely to be associated with clinical mastitis vs. subclinical mastitis and vice versa (Persson Waller et al., 2011; although, see Condas et al., 2017b) and resistance patterns of NASM are species-specific (see below), this observed difference in AMR prevalence between sample type may ultimately result from species differences between the 2 categories. In Persson Waller et al. (2011), *S. epidermidis* and *S. saprophyticus* were more prevalent in subclinical vs. clinical mastitis, while *S. hyicus* was more common in clinical mastitis. The authors attribute the higher proportion of penicillin resistance in subclinical isolates to the high prevalence of *S. epidermidis* and *S. saprophyticus* in these samples, as these species demonstrated significantly more penicillin resistance when compared with other NASM. Further support that differences in AMR for NASM associated with clinical vs. subclinical mastitis is primarily a result of species differences is found in Naushad et al. (2018). In their analyses of 328 NASM isolates from samples with subclinical mastitis and 57 isolates from clinical mastitis, within the same species, no significant differences existed in the prevalence of drug-specific AMR or resistance determinants when contrasting the two sample types. The importance of differentiating between species with broad groups of mastitis pathogens has also been highlighted by Sweeney et al. (2024) for the streptococci, and recent advances to discriminate the major mastitis pathogens *Streptococcus dysgalactiae* and *S. uberis* from other organisms grouped among the “environmental streps” (e.g. *Enterococcus* spp. and *Aerococcus* spp.).

Other factors that might influence susceptibility test results in prior surveys include the inclusion of isolates that have been treated and failed treatment versus isolates from cases prior to treatment, or quantification of antimicrobial use on the farms of origin. A few studies have accotuned for these variables in their design (Ruegg studies).

***Summary of studies describing AMR of staphylococci from conventional vs. organic dairies***

Nomenclature for the group of staphylococci causing bovine IMI excluding *S. aureus* has shifted over the past few decades, as both phylogeny and techniques for species-level identification have evolved. Some species which had been previously identified as staphylococci were recognized more recently as belonging instead to a closely related genus (*Mammaliicoccus*), and identification methods beyond a coagulase test have become more widely used. Although NASM is used throughout the rest of the review, the terminology used below when referring to results of a specific study is consistent with authors’ language and groupings of organisms (e.g., “coagulase-negative staphylococci,” or “CNS;” “non-*aureus* staphylococci,” or “NAS”, and staphylococci now grouped in the Mammaliicoccus genus, e.g. previously *S. sciuri, S. fleurettii, S. vitulinus*). This decision was made in an attempt to be consistent with the original authors’ contemporary understanding of phylogeny and methodology, as well as the methods of species identification (e.g., where coagulase testing was included in the study).

Overall, studies comparing AMR of mastitis-associated staphylococci between herds under organic management and herds managed conventionally find either no difference or that isolates originating from organic farms exhibit slightly more susceptibility (Table 1). However, these studies vary widely in their approach to exploring this question, primarily in number of isolates included and herds sampled, as well as approach to statistical analysis. In a descriptive study from Switzerland, Busato et al. (2000) found that the proportions of *S. aureus* isolates from organic herds (ORG) resistant to different antimicrobials were equivalent to those from conventional herds (CON). Similarly, the proportions of resistant isolates of CNS were comparable between the two systems, with the exception of a numerically higher proportion resistant to rifamyin from organic herds. A limitation of this study is that the data describing susceptibility of staphylococci from conventional herds was from a previously unpublished survey by the authors, and not contemporaneous with analysis of the organic isolates. In another descriptive study, researchers in Norway (Garmo et al., 2010) found similar proportions of *S. aureus* and CNS isolates resistant to penicillin between the two herd types (*S. aureus:* 6/68 or 8.8% from CON, vs. 9/64 or 14.0% from ORG; CNS: 81/167 or 48.5% for CON, vs. 93/200 or 46.5% from ORG). The authors note that penicillin resistance was proportionately higher in CNS vs. *S. aureus* isolates, consistent with more recent work looking at the resistance of staphylococci from bovine milk samples (as summarized in Taponen et al., 2023). In a Swiss study comparing resistance profiles of NAS and *S. aureus* from quartermilk samples, Roesch et al. (2006) also found that NAS isolates exhibited a higher overall percentage of AMR than *S. aureus* isolates. For 12 antimicrobials representing either drugs used to treat mastitis in dairy herds or drugs important in human medicine, they found that percentage of AMR did not differ significantly between *S. aureus* and NAS isolates from cows kept on organic vs. conventional herds. Although the overall proportion of *S. aureus* isolates resistant to ≤1 antimicrobial was numerically higher from organic cows (16/46, 35%) vs. conventional cows (6/33, 18%), this difference was not statistically significant. The proportion of NAS isolates resistant ≤1 antimicrobial to between systems was very similar (ORG: 9/19, 47%; CON: 10/19, 53%).

In contrast, Bombyk et al. (2008) found that staphylococci causing mastitis on organic dairies were associated with more overall antimicrobial susceptibility than those from conventional farms. For this study, researchers differentiated mastitis-associated staphylococci into 3 categories: coagulase-positive *Staph.* (CPS), novobiocin-sensitive CNS (NSCNS), and novobiocin-resistant CNS (NRCNS). In an analysis combining all 3 groupings of staphylococci, a larger proportion of isolates from organic herds were susceptible to pirlimycin and tetracycline compared with those from conventional herds. Susceptibility to erythromycin and penicillin did not differ significantly by herd type when all staphylococci were combined (CON vs. ORG). No significant differences between organic and conventional systems were found for *S. aureus*, although the numbers of isolates found was fairly small compared to both categories of CNS (36 *S. aureus* vs. 210 NSCNS and 159 NRCNS). When each category of CNS (novobiocin-susceptible or resistant) was analyzed separately, isolates within both groups from organic herds were more likely to be susceptible to pirlimycin than CNS from conventional dairies. No difference in tetracycline, erythromycin or penicillin susceptibility was seen between herd types (CON vs. ORG) within either CNS category. A larger proportion of NSCNS vs. NRCNS (when analyzed separately for conventional and organic herds) were susceptible to tetracycline, leading the authors to suggest that management practices unrelated to antimicrobial use may contribute to the observed differences in susceptibility patterns of CNS on dairy herds.

A number of studies comparing resistance patterns of mastitis-associated bacteria between conventional and organic dairy systems have focused specifically on *S. aureus.* Researchers in New York and Vermont (US) found that *S. aureus* isolates from both types of herds showed good susceptibility to most antimicrobials used to treat mastitis, but isolates from organic herds were significantly more susceptible (Tikofsky et al., 2003). In this study, researchers took two different approaches to analyzing the data: 1) the strength of association between the proportion of susceptible and resistant isolates was evaluated by management category, and 2) numeric differences in mean zone diameter were compared for isolates from organic vs. conventional herds. When results were combined over both analyses, *S. aureus* isolates from organic herds were more susceptible than those from conventional herds for 7 of the 9 antimicrobials studied. Contrary to these findings, researchers comparing resistance of isolates from bulk tank milk of organic and conventional systems in both the US and Denmark found that overall, antimicrobial susceptibility was very similar for *S. aureus* in both countries (Sato et al., 2004). Bulk tank isolates from conventional herds in Wisconsin (US) had significantly reduced susceptibility to ciprofloxacin (vs. isolates from organic herds), and isolates from organic herds in Denmark had reduced susceptibility to avilamycin (vs. isolates from conventional herds). In a finding highlighting the importance of geography in epidemiological studies, authors point out that differences in the antimicrobial susceptibility of *S. aureus* isolates between organic and conventional herds were small relative to differences in resistance patterns observed between countries. In agreement with Sato et. al, Bennedsgaard et al. (2006) observed no statistically significant differences in the prevalence of cows with penicillin-resistant *S. aureus* mastitis or the proportion of *S. aureus* isolates from quartermilk resistant to penicillin between conventional and organic dairies in Denmark.

Two studies looking at bulk tank milk (BTM) focused on detection of staphylococci carrying genetic determinants conferring methicillin resistance (*mecA* and *mecC* genes), an important consideration for public health globally. In a large study with the goal of surveilling dairy-associated methicillin-resistant *S. aureus* (MRSA)in Germany, researchers collected BTM from 372 conventional and 303 organic herds (Tenhagen et al., 2018). Using binary logistic regression to describe association of MRSA-positive samples with herd type (conventional vs. organic), they found that the prevalence of MRSA was significantly higher in BTM samples from conventional herds (9.7%) compared with organic herds (1.7%). The model-based approach allowed researchers to control for the effects of geographical region and herd size, both of which were also significant predictors of MRSA herd status. When comparing the proportion of BTM MRSA isolates resistant to 12 different antimicrobials between conventional and organic herds, MRSA isolates from conventional farms tended to be more resistant. However, as there were a limited number of isolates from organic herds (n = 5) compared to conventional herds (n = 36), no statistical analyses were performed. A large, multistate study in the US sampled BTM from 192 organic herds and 100 conventional herds matched for geographical location and herd size (Cicconi-Hogan et al., 2014). They identified 13 isolates from BTM as methicillin resistant (*mecA*-positive): 7 isolates from conventional herds and 6 from organic. Using 16S rRNA and *rpoB* genes for species-level identification, these 13 isolates were identified as *S. aureus* (n = 1), *S. sciuri* (n = 5), *S. chromogenes* (n = 2), *S. saprophyticus* (n = 3), *S. agnetis* (n = 1), and *Macrococcus caseolyticus* (a genus closely related to staphylococci; n = 1). The single methicillin-resistant *S. aureus* isolate was from an organic herd, for an observed 0.3% prevalence of MRSA at the herd level. Methicillin-resistant CNS were found at a prevalence of 2% in the organic population and 5% in the conventional population. The authors highlight the relatively large number of methicillin-resistant *S. sciuri* identified (6 out of the 12 methicillin-resistant CNS) compared with previous work, and also suggest that a potential methicillin-resistant *Staphylococcus* reservoir in the dairy herd population of the US may be independent of the type of production system. To this point, Walther and Perreten (2007) report the occurrence of a dairy cow on an organic farm in Switzerland that was diagnosed twice within 2 months with subclinical mastitis caused by methicillin-resistant *S. epidermidis*. The two strains had identical PFGE patterns of chromosomal DNA, exhibited resistance to chloramphenicol, and contained streptomycin- and trimethoprim-resistance genes but did not display phenotypic resistance against these drugs *in vitro*. Furthermore, the second *S. epidermidis* isolate contained an additional aminoglycoside-resistance gene, indicating the potential acquisition of resistance by horizontal gene transfer since isolation of the first bacterium. Similar to Cicconi-Hogan et al. (2014), the authors highlight that this finding demonstrates cows on organic farms may harbor multidrug-resistant staphylococci despite the limited use of antimicrobials under EU organic regulations.

Perhaps a limitation of the above studies comparing the resistance of staphylococci from organic and conventional dairy farms is that limited or no quantification of on-farm antimicrobial usage was calculated or presented. In order to evaluate if the level of antimicrobial usage in food animals selects for drug-resistant pathogens, an important component in a study exploring this question would be a quantification of antimicrobial use at the farm or cow level to be able to estimate the amount of selective pressure exerted on intramammary pathogens. Although all antimicrobial usage is prohibited on US organic dairies, the amount and type of antimicrobials used by conventionally-managed farms can vary widely (Pol and Ruegg, 2007b). Two of the largest-scale, statistically robust studies comparing the resistance profiles of staphylococci from quartermilk samples between conventional and organic dairies include a detailed, numeric quantification of antimicrobial usage by enrolled farms. In a 2007 study in the US, Pol and Ruegg report a standardized level of exposure to 10 different antimicrobials by calculating of the number of defined daily doses used per cow on each enrolled farm, and then categorize the 40 enrolled herds based on their respective antimicrobial usage. Herds are categorized into 3 groups: organic (no antimicrobial usage), conventional–low usage (conventional farms not using or using ≤ the first quartile of use for each drug; CON-LO), and conventional–high usage (conventional farms using > the first quartile for a particular drug; CON-HI). The authors took multiple approaches to compare resistance among isolates from the 3 antimicrobial usage groups. First, they compared the proportion of each type of isolate (CNS or *S. aureus*) that was susceptible or resistant in each category (CON vs. ORG) using a categorical test of association, in order to explore if proportion of susceptible isolates was independent of herd type. Secondly, they used a test of association to explore if the MIC for each type of isolate (CNS or *S. aureus*) was independent of herd type (CON vs. ORG). Lastly, they performed survival analysis for each type of isolate (CNS or *S. aureus*) based on the 3 antimicrobial usage categories (ORG, CON-LO, or CON-HI). In this last analysis of “time to event,” antimicrobial concentration in wells of the susceptibility test was considered “time,” and the “event” was inhibition of any bacterial growth. Overall, Pol and Ruegg found that isolates from organic herds were more susceptible to antimicrobials than those from conventional herds. Specifically, for *S. aureus*: (1) isolates from conventional herds were more likely to be resistant to ampicillin and penicillin when compared with isolates from organic herds, and herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested; (2) isolates from conventional herds had a higher MIC for pirlimycin and sulfadimethoxine compared with isolates from organic herds, and herd type was not associated with the MIC of the other antimicrobial drugs tested; and (3) in the survival analysis, the MIC that inhibited 90% (MIC90) of *S. aureus* isolates from organic herds for penicillin and pirlimycin was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (MIC50, the MIC that inhibited 50% of isolates, was not different for these drugs). For CNS: (1) isolates from conventional herds were more likely to be resistant to ampicillin, penicillin, pirlimycin, and tetracycline compared with isolates from ORG herds, and herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested; (2) isolates from conventional herds had a higher MIC for ampicillin, pirlimycin, and tetracycline compared with isolates from organic herds, and herd type was not associated with the MIC of the other antimicrobial drugs tested; and (3) in the survival analysis, the MIC90 of CNS isolates from organic herds for ampicillin, penicillin, pirlimycin, and tetracycline was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (ORG and CON-LO herds had a lower MIC50 for erythromycin than CON-HI herds, but the MIC90 did not differ by usage group). The authors highlight that although some differences were found between antimicrobial usage groups, most isolates from all farm types were inhibited at the lowest dilution tested of most antimicrobial drugs routinely used on dairy farms.

The other study comparing resistance of staphylococci between organic and conventional dairies to include a detailed quantification of antimicrobial usage enrolled 7 organic herds, 11 conventional herds using ampicillin-cloxacillin dry cow therapy (CON-AC), and 8 conventional herds using cephalonium dry cow therapy (CON-CE) in New Zealand (McDougall et al., 2021). Although the study was carried out in NZ, participating herds were all certified under the USDA National Organic Program. Conventional herds of both categories were selected on the basis that >50% of the cows were treated in each of the 3 previous years with at least 1 dry cow therapy (DCT) product. Similar to Pol and Ruegg (2007a), the authors took a multifaced approach to exploring the resistance patters of *S. aureus* and CNS from organic and conventional systems. Overall, the MIC of CNS from ORG herds were lower than isolates from both types of CON herd. For *S. aureus,* they found that the MIC50 for ampicillin and penicillin were greater by more than 1 dilution for isolates from CON-CE herds compared with CON-CA and ORG herds, but this relationship did not hold for the MIC90 of these drugs (MIC for CON-CE and ORG herds was greater than that for CON-CA herds).In a univariate analysis, the proportion of penicillin-resistant *S. aureus* isolates was significantly higher in CON-CE herds (76/111; 68.5%) compared to CON-CA (4/99; 4.0%) or ORG herds (32/110; 29.1%). A multilevel model (accounting for clustering of quarter within cow within herd) was made, where the 3 herd types were the main explanatory variable. Other potential variables offered to this model included age of the cow, breed, DIM at time of sampling, SCC at last test, and antimicrobial treatment history for that cow.Results from this multilevel model showed that the proportions of penicillin-resistant *S. aureus* isolates did not differ between the 3 herd types. For analysis of resistance to ceftiofur, sulfadimethoxine, and erythromycin, 3 different groupings of breakpoints were made for each compound.When comparing the proportion of *S. aureus* isolates falling into the 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were fewer organic isolates in the middle breakpoint category (1 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.When comparing the proportion of *S. aureus* isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance, the only significant difference was that there were more organic isolates in the lowest category (32 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.There were no significant differences between the 3 herd types when comparing the proportion of *S. aureus* isolates falling into 3 different breakpoint groups for erythromycin resistance. For CNS isolates, the MIC50 and MIC90 for ampicillin and penicillin were lower by more than 1 dilution for CNS isolates from organic herds compared to both types of conventional herds; otherwise, these values did not differ by more than 1 dilution between the 3 herd types for the other antimicrobials tested.In a univariate analysis, the proportion of penicillin-resistant CNS isolates was significantly greater in both types of conventional herds (CON-CE, 42/82; 51%; CON-CA, 22/74; 30%) vs. organic herds (14/84; 17%). Similar to the analyses for *S. aureus,* a multilevel model was made to compare penicillin resistance of CNS with herd type as the main explanatory variable. Results from this multilevel model showed that the proportion of penicillin-resistant CNS isolates was significantly greater for CON-CE herds (0.50 ± 0.07) compared to CON-CA (0.31 ± 0.06) or ORG herds (0.17 ± 0.05).When comparing the proportion of CNS isolates falling into 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were more organic isolates in the lowest (0.5 μg/mL) and highest (2 μg/mL) categories compared to both conventional herd types; otherwise, there were no differences in the proportion of isolates falling into the various breakpoint groups from each of the 3 herd types.There were no significant differences between the 3 herd types when comparing the proportion of CNS isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance.When comparing the proportion of CNS isolates falling into 3 different breakpoint groups for erythromycin resistance, the only significant difference was that there were more CON-CA isolates in the highest category (≥1 mg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoints from each of the 3 herd types.Importantly, the authors point out that any differences in MIC between isolates from different herd types occurred below clinical breakpoints, so therefore may not affect bacteriological cure rates. Rather unexpectedly, they found bimodal distributions of MIC for ampicillin and penicillin in *S. aureus* isolates from organic herds, suggesting either (1) isolates with a higher MIC are “a natural part of the bacterial population of the bovine mammary gland,” or (2) isolates with higher MIC have persisted within organic herds from a time when antimicrobials were used on the farm. One factor not included in these prior studies was strain typing of the isolates. Chakrawarti et al (2024) reported the association between *S. aureus* strain types and antimicrobial susceptibility among isolates collected from cattle and humans on organic and conventional farms in Vermont. Human associated *S. aureus* strain types were more likely to be phenotypically resistant and carry genetic resistant markers, consistent with other reports describing associations between strain type and antimicrobial susceptibility. Thus, a third possibility from the work of McDougall et al. (2021) is there is potential spillover and host switching of isolates from other sources (such as humans or other host species) to cows in these farm systems. Nobrega et al., (2018) demonstrated that among the non-aureus staphylococci antimicrobial resistance was species dependent. These studies reinforce the concept that among the staphylococci and closely related species (SaM) antimicrobial resistance may be associated with species and strains, so studies characterizing susceptibility among the staphylococci should differentiate among species and where feasible strains.

Dairy farms in the process of transitioning from conventional management to organic certification provide a unique opportunity to study patterns resistance over time after a change in the level of antimicrobial exposure. In addition to comparing conventional and organic farms, Bennedsgaard et al. (2006) followed 19 Danish herds in the process of transitioning to becoming certified organic dairies. These herds were sampled at year 0, 1, and 2 of transition, with quartermilk samples collected from 30 cows at each farm at high risk of infection with *S. aureus* (as determined by a score based on a history of high SCC, breed, and lactation). Herds in the “old organic” category were certified for ≥ 5 years. Antimicrobial exposure for each herd was approximated by calculating the amount of mastitis treatments used in % cows treated/cow-year. The amount of mastitis treatment used by the conventional group was significantly higher than “old organic” herds, but no other significant differences existed between “old organic” herds or the conventional herds in comparison to any of the transition groups (transition year 1, transition year 2, transition year 3) with respect to usage of antimicrobial mastitis treatments. As previously mentioned, the prevalence of penicillin resistance in *S. aureus* and the proportion of penicillin-resistant isolates was similar between “old organic” and conventional herds. Furthermore, no differences were seen in these measures of penicillin resistance between “old organic,” conventional, or any of the 3 transition groups. The same 19 herds were sampled repeatedly over 3 years, and the amount of penicillin resistance among *S. aureus* on these farms did not decrease year after year as they transitioned to organic status. This finding is somewhat unsurprising in light of the fact that antimicrobial usage also was not significantly different. In contrast, Park et al. (2012) found that β-lactam resistance rates of CNS decreased with discontinuation of β-lactam antibiotics in a study following 2 dairies through the process of converting from conventional to organic management over a 3-year period. Composite milk samples were collected from cows at the end of lactation, at freshening, and from cases of clinical mastitis during the last year of conventional dairy production, the transition year, and during the first year of organic production. While still conventional, cows with clinical mastitis were treated with an intramammary product with pirlimycin, and a product with cephapirin, streptomycin and penicillin, or novobiocin and penicillin was given to all cows at dry-off. There was a significant increase in zone diameter for mastitis-associated CNS isolates against cephalothin, cloxacillin, and penicillin when comparing the conventional vs. organic phase. There was no significant change in zone diameter of the other 8 antimicrobials tested. Interestingly, no changes in resistance patterns were seen for mastitis-associated *S. aureus* isolates for the 12 antimicrobials tested. Of importance to note is that the 2 farms in Park et al. were in the US, and therefore antimicrobial usage was completely discontinued at the beginning of the transition to organic status. A similar small-scale case report from Thailand compared AMR of mastitis pathogens before and after the experimental farm’s transition from conventional to organic status for 7 antimicrobial drugs used to treat mastitis (Suriyasathaporn, 2010). All cows in the herd were sampled before beginning the transition, and after 6 months of operating as an organic dairy. The frequency of antimicrobial treatment on the farm decreased from <3 cases/month to > 1 case/month during the study period. Although isolate numbers were small (7 CNS isolates from before transition, 6 from after), a significant decrease was seen in the percent of CNS isolates resistant to gentamycin. Although numeric decreases in percent of resistant CNS isolates were seen for the other 6 antimicrobials, no changes were statistically significant. Data on susceptibility was not reported for *S. aureus* isolates.

***Additional factors explaining variation in antimicrobial susceptibility of staphylococci***

Although some evidence exists that conventional vs. organic management may influence the prevalence of AMR in staphylococci causing bovine IMI, this relationship is difficult to tease out from other factors determining the resistance profiles of these mastitis pathogens. This is especially true for NASM (primarily grouped as “CNS” in these studies), where prevalence and type of AMR carriage differs by species. Herd-level management factors, cow-level factors, and geography have all been shown to influence which NASM species may be present or predominant in causing IMI in a particular herd (see below). It is therefore difficult to attribute differences in AMR prevalence of NASM without accounting for this species-level effect. Table 2 summarizes work describing the species-specific antimicrobial susceptibility of staphylococci isolates from bovine IMI. The 10 observational studies included describe phenotypic resistance profiles and are limited to work where isolates were identified to species level using genotypic techniques or MALDI-TOF.

When considered as a group, resistance to β-lactam antibiotics is the predominant type of AMR present in staphylococci. The reported proportion of NASM isolates with β-lactamase resistance can be fairly high, with 51.6% phenotypically resistant to penicillin in Argentina (Raspanti et al., 2016), 63% phenotypically resistant to penicillin in South Africa (Phophi et al., 2019), and 80% of CNS isolates positive for the *blaZ* gene (encoding the production of a β-lactamase enzyme) in a study from the Netherlands (Sampimon, 2009). Proportion of phenotypically penicillin-resistant NASM seems to vary geographically, with Nordic countries reporting 34% (Nyman et al., 2018), 23% (Fergestad et al., 2021), and 29% (Persson Waller et al., 2011), while a Korean study found 14% of NASM isolates were resistant to penicillin (Kim et al., 2019) and Nobrega et al. (2018) report a prevalence of 10% in Canada. β-lactam antibiotics are among the few choices for treating mastitis in the US, with first- and third-generation cephalosporins being the most commonly-used mastitis treatments (USDA, 2016; de Campos et al., 2021). Moderate resistance has been observed in NASM against tetracycline, another highly important antimicrobial frequently used in dairy herds, with 30.1%, 20.9%, and 10% of isolates reported to be resistant in Argentina, India, and Canada, respectively (Raspanti et al., 2016; Mahato et al., 2017; Nobrega et al. 2018). This marked geographic variation in resistance patterns may likely be due to differing selective pressure in dairy farm systems around the world. Which specific antimicrobials are most typically used to treat mastitis and in what amount, as well as the various regulation around their usage, varies from country to country.

Studies comparing NASM at the species level have consistently shown that AMR profile varies between species (Sampimon, 2009; Persson Waller et al., 2011; Taponen et al., 2016; Nobrega et al., 2018; Fergestad et al., 2021; Taponen et al., 2023). Overall, both phenotypic resistance and resistance genes are relatively rare in the most common species, *S. chromogenes,* in comparison to other NASM (Sampimon, 2009; Persson Waller et al., 2011). A notable exception is the presence of the *blaZ* gene, which was found in 80% of all 170 CNS isolates and 87% of *S. chromogenes* specifically in a Flemish study (Sampimon, 2009). β-lactamase production was significantly lower for *S. chromogenes* vs. *S. epidermidis* and *S. haemolyticus* in Sweden (Persson Waller et al., 2011). Although a smaller-scale study in Argentina found a relatively high proportion of *S. chromogenes* were resistant to penicillin (45.1%), both *S. haemolyticus* and *S. xylosus* had an even higher proportion of penicillin-resistant isolates (58.6% and 92.9%, respectively; Raspanti et al., 2016). Across a number of studies, authors report that some less-commonly isolated NASM species carried AMR profiles which were the most concerning for public health. Sampimon et al. (2011) found a high prevalence of genotypic resistance (particularly *mecA*) or presence of multiple resistance genes in species with relatively a low prevalence (*S. cohnii, S. equorum, S. fleurettii,* and *S. sciuri*). In Nobrega et al. (2018), resistance to quinupristin/dalfopristin (a combination used to treat serious nosocomial infections in humans) was common in *S. gallinarum* (98% prevalence of resistance among isolates), and *S. cohnii* and *S. arlettae* were frequently resistant to erythromycin (prevalence of 63 and 100%, respectively). The authors specifically highlight *S. arlettae* as worrisome in its AMR profile; it had the highest prevalence of AMR against penicillin (61%), ampicillin (23%), erythromycin (100%), pirlimycin (18%) and clindamycin (99.9%), as well as the highest prevalence of multidrug resistance. A number of studies also call attention to concerning AMR patterns for *S. epidermidis,* which is moderately common in the US and Canada but one of the predominant species found in Nordic countries. In Sampimon et al. (2009), *S. epidermidis* was the second most commonly-found species, it carried multiple resistance genes in ~50% of isolates, and phenotypic penicillin resistance was more common compared to other CNS. The proportion of penicillin-resistant isolates was highest for *S. epidermidis* in a Finnish study compared to other species, with *S. epidermidis* accounting for 6/8 NASM isolates carrying the *mecA* gene (Taponen et al., 2023). Similarly, β-lactamase production was higher for *S. epidermidis* compared to other species (Persson Waller et al., 2011), and itwas one of a few species where AMR (including resistance to trimethoprim-sulfonamide) was most frequently observed in Fergestad et al. (2021). Lastly, Taponen et al. (2016) found that *S. epidermidis* was the most resistant among the four major species studied, several isolates were multidrug resistant, and 19% of isolates were *mecA*-positive (encoding for methicillin resistance). Even within a given species, AMR carriage has been linked to certain strain types. For *S. aureus*, carriage of methicillin resistance has been associated with particular clonal complexes both in human medicine (Smith et al., 2021; Garrine et al., 2023) and certain clusters of *spa* ­type for bovine clinical mastitis isolates (Freu et al., 2022). The linkage between strain type and AMR is not as well studied for NASM, but Persson Waller et al. (2023) found that *blaZ* was significantly more common among *S. chromogenes* strains belonging to 2 specific clusters of strain types vs. strains belonging to other clusters.

As AMR carriage differs by species, the particular diversity of NASM responsible for causing IMI on a farm will partly determine the observed herd-level resistance pattern, especially if the NASM are group without differentiating among species. Various regional and herd-level risk factors have been identified explaining some of the diversity and prevalence of different NASM associated with mastitis and BTM. Different times of year were associated with higher likelihood of IMI for *S. chromogenes, S. haemolyticus, S. xylosus,* and *S. warneri* in Dolder et al. (2017), and *S. cohnii, S. simulans, S. sciuri* in BTM in De Visscher et al. (2017). Geographical differences in NASM species diversity among quartermilk samples were found between 4 regions in Canada (Condas et al., 2017a) and 4 states in the US (Jenkins et al., 2019). It is difficult to discern whether these differences are truly a function of geographical variation, or result from farms in a region sharing a similar suite of management practices leading to similar NASM species prevalence and diversity in a herd. Although *S. chromogenes* is the dominant species causing IMI in many countries (as summarized in De Buck et al., 2021), *S. epidermidis* (closely followed by *S. simulans*) was the most commonly-found species in both a Finnish (Taponen et al., 2022) and a Swedish study (Nyman et al., 2018). At the herd level, among Canadian herds, facility type has been shown to explain some of the diversity of NASM species: cows from herds using a tiestall barn were more likely to have an IMI due to *S. simulans*, *S. xylosus, S. cohnii, S. saprophyticus, S. capitis,* and *S. arlettae* compared with other NASM species, and less likely to have an IMI due to *S. epidermidis* (Condas et al., 2017a)*.* Cows from herds in Canada using a bedded pack system had a higher relative risk for IMI due to *S. chromogenes* and *S. sciuri* vs. other NASM (Condas et al., 2017a), while Adkins et al. (2022) found *S. cohnii*, *S. hyicus*, and *S. pseudintermedius* in BTM from sand-bedded freestalls (but not bedded packs), and *S. pasteuri* and *S. piscifermentan*s were unique to BTM from bedded packs. In a study by Piessens et al. (2011), sawdust bedding material was associated with IMI due to *S. xylosus* and *S. succinus* for Belgian dairy herds. De Visscher et al. (2017) identified a number of management practices around milking protocol and hygiene associated with the presence of different NASM species in BTM. These include a decreased risk for *S. xylosus, S. simulans,* and *S. chromogenes* in BTM from herds that clip udders, a decreased risk of *S. devriesei* in herds with consistent glove use during milking, an increased likelihood of *S. cohnii* in herds sharing towels between cows when drying udders, and a decreased likelihood of *S. haemolyticus, S. cohnii,* and *S. simulans* in herds that flushed or steamed milking units after use. Hogan et al. (1987) found more IMI due to *S. epidermidis* in herds using no teat dip compared to herds that did, and that *S. hyicus* constituted a greater proportion of staphylococci IMI in herds that used teat dip vs. herds that did not. However, it should be noted that species-level identification of staphylococci in this study was performed using a biochemical test, which may have had limited typeability and accuracy for identification of bovine staphylococci isolates (Vanderhaeghen et al., 2015). Lastly, some herd-level management factors associated with NASM diversity were related to feed and water provided to dairy cows: De Visscher et al. (2017) found an increased likelihood of *S. simulans* in BTM if drinking water for cows was from a public supply (vs. a well), and Petzer et al. (2022) reported proportionally more IMI due to *S. chromogenes* from herds that were pasture-based compared to those that were fed a total mixed ration (TMR), while *S. haemolyticus* was more likely to cause IMI for TMR herds.

Risk factors at the cow level which affect the likelihood of IMI with different NASM have also been identified. Both Thorberg et al. (2009) and Mork et al. (2012) found that *S. chromogenes* was more likely to be isolated from first-lactation animals, while *S. epidermidis* was found more often in third-lactation and older cows. These findings are consistent with 3 other studies reporting *S. chromogenes, S. xylosus,* and *S. simulans* more commonly caused IMI in heifers vs. third-lactation and older cows (De Visscher et al., 2016; Condas et al., 2017a; Nyman et al., 2018). The most likely species to cause IMI also varies within a lactation: Dolder et al. (2017) found that *S. xylosus* was more commonly found in early lactation and *S. warneri* was isolated from mid- to late-lactation animals, while Condas et al. (2017a) report the prevalence of *S. chromogenes, S. gallinarum, S. cohnii,* and *S. capitis* to be highest at freshening, and the prevalence of *S. chromogenes* (after an initial decrease from levels at freshening)*, S. haemolyticus, S. xylosus,* and *S. cohnii* increased throughout lactation. In Belgian herds, *S. chromogenes* was the predominant species causing IMI both at parturition and throughout lactation; the next most commonly seen species at freshening were *S. sciuri* and *S. cohnii* (De Visscher et al., 2016), while *S. simulans, S. xylosus, S. epidermidis,* and *S. haemolyticus* were the next most common causes for NASM IMI during lactation (Piessens et al., 2011; Supré et al., 2011). Dirty teats have been associated with an increased likelihood of IMI due to *S. cohnii, S. equorum, S. saprophyticus,* and *S. sciuri,* which the authors indicate is consistent with a likely environmental origin for these species (De Visscher et al., 2016). Even physical features of the udder and teats have been associated with different NASM species (De Visscher et al., 2016: quarters with an inverted teat end had higher odds of being infected with *S. chromogenes, S. simulans,* or *S. xylosus*; Dolder et al., 2017: udder edema was a risk factor for IMI with *S. chromogenes*).

In addition to unmeasured animal or management-associated risk factors, an important determinate in AMR carriage of mastitis isolates is clonal dissemination within a particular herd. Consistent with behavior of a contagious mastitis pathogen, a certain strain (or strains) of *S. aureus* will predominant for any given herd (Lange et al., 1999; Zadoks et al., 2000; Freu et al., 2022). If the dominant strain of *S. aureus* causing IMI in a dairy herd happens to carry a given AMR determinant, a high proportion of *S. aureus* isolates from that herd will likely exhibit phenotypic resistant against a particular antimicrobial: not solely as a result of environmental pressure and selection, but also as a consequence of phylogeny and the behavior of the pathogen itself. This was observed by Barlow et al., (2013) in a 13-month longitudinal study where in one conventional herd 8 of 18 *S. aureus* subclinical IMI were beta-lactam resistant and blaZ positive, with 7 of these resistant isolates belonging to the same strain type (ST8). This dominant strain type effect can result in issues of non-independence between isolates from a particular farm (Call et al., 2008), which would be exacerbated in studies enrolling a relatively small number of herds. Pol and Ruegg (2007a) directly address this issue of statistical dependence in their study of 40 herds. In order to avoid dependence between the cow, herd, and exposure category (conventional vs. organic), the authors included only 1 isolate per cow and ≤ 20 isolates per herd in all analyses. Additionally, they report the range of isolates used per herd for each category of mastitis pathogen.

***Why is AMR maintained in organic systems?***

In almost all studies summarized in this review, some degree of AMR was found in isolates despite decreased (EU) or absence (US) of selective pressure of antimicrobial use; organic farms in McDougall et al. (2021) had no antimicrobial usage for a range of 7-19 years, with a median of 12 years of organic certification. Assuming there is a fitness cost to bacteria for maintaining AMR genes (Vanacker et al., 2023), this certainly begs the question of why resistance genes are maintained to any degree in the absence of selective antimicrobial pressures. A rather extreme example of AMR persistence in cattle farms is a study comparing bacteria isolated from retail ground beef raised in conventional and “raised without antibiotics” operations. LeJeune and Christie (2004) identified resistance against chloramphenicol in isolates from both systems, an antimicrobial that had been banned from use in US food animals since 1986. Resistant bacteria remaining on organic farms long after selective pressure of antimicrobial use is gone suggests that other factors play an important role in this long-term persistence. In a study where feedlot steers were fed subtherapeutic levels of antibiotics, Alexander et al. (2008) found that ampicillin-resistant *E. coli* in the control group (no antibiotics) increased due to an evident clonal expansion of an environmental strain (detected by PFGE) during the latter part of this longitudinal study. This environmental strain outcompeted other strains of *E. coli* present in the intestinal tract of the steers in the control group, suggesting that fitness traits beyond carriage of AMR genes play an important role in the prevalence of AMR bacteria. Specifically, the authors suggest that one environmental factor related to the level of AMR was diet, as the prevalence of steers shedding tetracycline-resistant *E. coli* was higher in animals fed grain-based vs. silage-based diets in both treatment and control groups. Although specifically looking at commensal *E. coli* in dairy calves and not mastitis pathogens, one group of researchers set out to explore which factors beyond antimicrobial usage may explain the persistence of an *E. coli* strain (SSuT) in the GI tract which was resistant to streptomycin, sulfonamide and tetracycline (Khachatryan et al., 2004, 2006a, 2006b, 2008; as summarized in Call et al., 2008). Their first study asked if direct antimicrobial selection pressure was maintaining a high prevalence of SSuT *E. coli* strains in calves, and they found that it was not; a clinical trial showed that addition or removal of oxytetracycline from the diet had no effect on the prevalence of SSuT strains in fecal samples over a period of 3 months. Their next step was to ascertain if SSuT traits themselves provide a secondary but unrecognized fitness advantage to these particular strains of *E. coli* by generating null mutants for the SSuT traits (now susceptible to these antibiotics). On average, they found that the null mutant strains retained a competitive advantage over the other susceptible strains, and concluded that the specific genes conferring the SSuT phenotype were not responsible for providing any secondary fitness advantages. At some point between studies, the farm stopped feeding a medicated milk replacer. The researchers observed that after only a short time frame, the SSuT strain had suddenly declined in prevalence. This was unexpected, given that their previous work demonstrated that the SSuT strains had an obvious advantage compared to the susceptible strains. This unexplained decline prompted an additional study, which hypothesized that the milk supplement itself (comprised of dried milk powder, vitamin A and D) was somehow providing an advantage to the SSuT strains. When the milk supplement was reintroduced (both with and without tetracycline), the prevalence of SSuT *E. coli* strains nearly doubled for both groups of animals receiving the milk supplement vs. those that received none. This work highlights an example of a positive selective force (a dietary supplement) in a dairy farm system either directly or indirectly favoring strains of resistant *E. coli,* which was completely unrelated to antimicrobial exposure.

Call et. al (2008) summarize the 3 possible outcomes after exposure to antimicrobials in an individual animal produces a transient increase in AMR prevalence in a population of bacteria, as has been documented to occur in fecal bacteria. Once the negative selective pressure of antimicrobial usage is removed, the first possible outcome is subsidence of AMR in the population, assuming there is a fitness cost to maintaining the AMR traits. Alternatively, if there is no additional fitness cost to maintaining AMR, we would expect to see “eventual displacement in the face of natural turnover of clonal types at the level of individual animals.” A third possibility, as seen in the work from Khachatryan et al., is that there is no (or limited) change in the level of AMR prevalence after selective pressure from antimicrobials is removed. This could occur if AMR traits have been coupled with other some other locally beneficial traits which provide the bacteria possessing them an advantage in their specific environmental niche. Call et al. (2008) illustrate this with a hypothetical model illustrating the effect of antimicrobial exposure in an individual animal (Figure 1). First, a transient increase occurs in the relative number of resistant bacteria within a population after exposure to an antimicrobial. During this time of increased replication, there is an increased probability for a genetic event to occur which links AMR carriage to some other trait providing increased fitness in that specific environment. Organisms with the linked AMR carriage and locally advantageous trait survive better in the population, but in the absence of antimicrobial exposure, there is nothing to actively suppress the susceptible strains in the population. Although the relative proportion of bacteria with AMR may decline gradually over time, linkage of AMR to some other advantageous trait could also lead to a gradual increase or maintenance of a baseline prevalence of AMR, even in systems devoid of antimicrobial exposure. So far, work exploring this question has been limited to studying the effect of antimicrobials on resistant bacteria present in the GI tract of cattle. The potential exists for research focused on exploring why maintenance of AMR genes occurs in mastitis pathogens from organic dairies, years after the selective pressure of antimicrobial use has been removed.

***Conclusions***

Organic dairy systems provide a novel opportunity in which to identify the antimicrobial resistance patterns of mastitis pathogens experiencing decreased or no selective pressure from antimicrobial use. This narrative review aimed to summarize studies comparing antimicrobial susceptibility of bovine staphylococcal mastitis isolates on organic vs. conventional dairy farms. Numerous factors make direct comparisons of AMR results difficult between studies, including: use of various methods for antimicrobial susceptibility testing and continuously evolving or conflicting schemes for breakpoints; variation in sampling scheme (random vs. targeted sampling of cows, bulk tank milk vs. quartermilk samples, inclusion of isolates associated with clinical vs. subclinical mastitis); differing definitions of “organic” between herds in the EU (where antimicrobial usage is still allowed, but is more tightly regulated and limited) and the US (any animal treated with antimicrobials must leave the herd). Furthermore, studies including a limited number of herds may suffer from a lack of independence between observations. However, the overall conclusions from each study comparing the two different management systems are still informative, as long as the methodology is consistent within a study. Generally, studies comparing the resistance profiles of staphylococci associated with bovine milk samples show that isolates from organic farms are similar or slightly more susceptible to antimicrobials than those associated with mastitis on conventional farms. Although some level of resistance was observed against a number of antimicrobials important for veterinary medicine (cephalosporins, penicillin, tetracycline), overall resistance of mastitis-associated staphylococci is generally low and the most commonly-used mastitis treatments are still effective. A considerable amount of resistance for both NASM and *S. aureus* against penicillin has been described, but the majority of isolates in European and US studies remain susceptible.

Another factor influencing AMR of staphylococci causing mastitis at the herd level is the particular assortment of NASM causing IMI in a herd, as resistance profiles are species-specific. Consequently, different management factors (unrelated to antimicrobial usage) which affect the prevalence and species diversity of NASM on particular farms can indirectly affect the prevalence of observed AMR in a herd. Furthermore, as strain types within species can differ in likelihood of AMR carriage, AMR prevalence may also be a function of predominate strain type(s) in a given herd.

A consistent finding between all studies described was the persistence of resistant mastitis-associated staphylococci on dairy farms which had not used antimicrobials for many years. Some insight on this phenomenon may be gleaned from a theory put forth to explain the observed maintenance of AMR in fecal bacteria in cattle, despite the absence of antimicrobial use. In the transient expansion of a population of resistant isolates following antimicrobial treatment, the likelihood increases that an AMR gene can become linked with some other locally advantageous trait during replication. The selective advantage bestowed on the resistant bacteria could then lead to an increase in their relative abundance and maintenance of the AMR genes over the long-term, provided that the trait linked to AMR continues to afford a selective advantage.

The biggest limitation of most studies comparing resistance profiles of mastitis pathogens between organic and conventional farms is that staphylococci were not identified to the species level. Organisms were primarily grouped as either *S. aureus* or “coagulase-negative staphylococci.” Before MALDI-TOF became more widely available, accurate species-level identification of mastitis-associated staphylococci on a relatively large scale was prohibitively expensive and time-consuming. As resistance profile varies by species, additional work comparing AMR for NASM isolates (while controlling for species) may give further insight into whether resistance profiles differ between management systems for these bacteria. Comparison of predominant strain types within a given species causing IMI between organic and conventional farms could further our understanding of the complex interplay between phylogeny and selection pressures resulting from management factors on AMR of mastitis pathogens. Although researchers were studying fecal *E. coli* and not mastitis pathogens, Walk et al. (2007) found that phylogenetic groupings varied between organic and conventional dairies, suggesting there may be differences between lineages of *E. coli* in their ability or likelihood of acquiring resistance genes. Based on their findings, the authors conclude that “organic farming practices not only change the frequency of resistant strains but also impact the overall population genetic composition of the resident *E. coli*flora.” Additionally, few studies have described resistance patterns of mastitis pathogens before and after transitioning to organic status, and most were limited in both the number of herds enrolled and the amount of time farms were followed. Although likely logistically difficult and expensive, a long-term, larger study of farms transitioning from conventional to organic status would be incredibly valuable in understanding what types of AMR are maintained in organic dairy herds and for how long.

Fortunately, AMR in general remains relatively low in mastitis pathogens from dairy farms. Nevertheless, continued surveillance and further understanding of factors affecting resistance of staphylococci is warranted. Not only are they important pathogens affecting human health, staphylococci are the predominant group of bacteria responsible for mastitis in dairy animals globally. Understanding the complicated interplay of factors affecting AMR in bacterial populations on dairy farms is vital to making science-based decisions around regulations dictating antimicrobial usage. It is in the best interest of the dairy industry to maintain effective antimicrobial treatments that keep cows healthy, decrease animal suffering, minimize production expenses for livestock producers, and allow dairy cows to produce a high-quality product.

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***Tables and figures***

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| --- | --- | --- | --- | --- | --- | --- |
| Table 1**.** Summary of observational studies comparing antimicrobial susceptibility of staphylococci isolates between organically-managed (ORG) and conventionally-managed (CON) dairy herds. Most studies describe using a combination of morphology, Gram staining, coagulase and catalase test to identify bacterial isolates as *S. aureus* or non-*aureus* staphylococci (NAS)/coagulase-negative staphylococci (CNS). Additional methods for identifying staphylococci to the species level are identified where appropriate. DCT = dry cow treatment; SCC = Somatic cell count; MIC = Minimum inhibitory concentration | | | | | | |
| *Reference; Country*  *Organisms described1* | *Study design and sampling scheme* | *Herd selection considerations*  *Min. no. yr. ORG certified* | *Quantification of AM usage*  *Description of antimicrobials used on farms* | *Susceptibility method2*  *Antimicrobials tested* | *No. isolates tested* | *Selected results* |
| Busato et al., 2000; Switzerland (EU)  *S. aureus*, CNS | Longitudinal (2 herd visits/yr.: 1x on pasture, 1x in confinement); Performed CMT on each lactating cow in herd, quartermilk samples then collected from quarters with CMT >1; Isolates from subclinical mastitis | 152 ORG herds; Stratified random selection (by herd size and farm location by altitude) from herds agreeing to participate; num. herds selected within strata based on actual proportion of herds in each stratum of entire population of Swiss organic dairies  No. yr. ORG herds certified not provided | No quantification of AM usage  65% ORG herds regularly used AM DCT treatment (mostly β-lactam antimicrobials, combinations of β-lactams and other antimicrobials) | *Disk diffusion*  Ampicillin, cefalotin, chloramphenicol, ciprofloxacin, clindamycin, cloxacillin, cotrimoxacol, erythromycin, gentamicin, neomycin, penicillin, rifamycin, tetracycline | *S. aureus*: 37 ORG  CNS: 54 ORG | Data describing the proportion of staphylococci from CON herds resistant to different antimicrobials taken from a previously unpublished survey by the authors (completed 6 years prior).3  Proportions of *S. aureus* isolates from ORG herds resistant to different antimicrobials were similar to those from CON herds (no statistical comparison carried out).  Proportion of CNS isolates from ORG herds resistant to different antimicrobials were similar to those from CON herds, with the exception of a numerically higher proportion of isolates resistant to rifamyin from ORG herds (no statistical comparison carried out). |
| Tikofsky et al., 2003; US  *S. aureus* | Cross-sectional (1 visit/herd); Composite quartermilk samples from each lactating cow in herd; Not specified if isolates from clinical or subclinical mastitis | 22 ORG herds, 16 CON herds; Herds of similar size and geographic distribution selected; All CON herds used blanket DCT  ORG herds certified ≥ 3 yr. ("most much longer") | No quantification of AM usage  On CON herds, β-lactam antimicrobials used most commonly (amoxicillin and pirlimycin most common treatments administered during lactation, penicillin-novobiocin for DCT) | *Disk diffusion*  Ampicillin, cephalothin, erythromycin, novobiocin, oxacillin, penicillin, penicillin-novobiocin, pirlimycin, tetracycline, vancomycin | 261 *S. aureus*: 117 CON, 144 ORG | Strength of association between proportion susceptible/resistant and mgmt. category was evaluated, as well as differences in mean zone diameter for isolates from ORG vs. CON herds.  Differences in antimicrobial susceptibility were observed between *S. aureus* isolates from ORG and CON herds for 7 of 9 antimicrobials studied (results combined over both analyses). *S. aureus* isolatesfrom both types of herds showed good susceptibility to most mastitis antimicrobials, but isolates from ORG herds were significantly more susceptible. |
| Sato et al., 2004; US and Demark (EU)  *S. aureus* | Cross-sectional (1-2 herd visits/yr. for US herds, 1 visit/herd for Danish herds); Bulk tank milk | 30 ORG herds, 30 CON herds from US; 20 ORG herds, 20 CON herds from Denmark; In US, "neighboring" CON herd enrolled as match for each ORG herd; Danish herds chosen randomly  US: ORG herds certified ≥ 3 yr. (mean = 8 yr.); Denmark: ORG herds converted ≥ 9 yr. prior to publication date | No quantification or description of AM usage provided | *Broth microdilution (Sensititre)*  Bacitracin, cephapirin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, oxacillin, penicillin, streptomycin, sulphamethoxazole, quinupristin/dalfopristin, tetracycline, trimethoprim, vancomycin | 483 *S. aureus*: 229 CON, 254 ORG | Overall, antimicrobial susceptibility was very similar between *S. aureus* isolates from ORG and CON herds in both countries. Isolates from CON herds in Wisconsin had significantly reduced susceptibility to ciprofloxacin (vs. isolates from ORG herds), and isolates from ORG herds in Denmark had reduced susceptibility to avilamycin (vs. isolates from CON herds). Differences in antimicrobial susceptibility of *S. aureus* isolates between ORG and CON herds were small relative to differences in isolates observed between the US and Denmark. |
| Bennedsgaard et al., 2006; Denmark (EU)  *S. aureus* | Cross-sectional and longitudinal components; Herds converting to organic farming sampled 3x 1 year apart, CON and ORG herds sampled 1x; Quartermilk samples collected from 30 cows with "high risk of infection" (criteria: history of high SCC, breed, and lactation); Not specified if isolates from clinical or subclinical mastitis | 20 CON herds, 18 ORG herds, and 19 transitioning herds (sampled at 0, 1, 2 yr. of transition); Herds not matched  ORG herds certified ≥ 5 yr. | Estimated mastitis treatments given in % cows treated/cow-year for each of 5 herd grps  CON used more than ORG, but transitioning grps not different from either CON or ORG; Type of AM usage not described | *Blood agar plates with 1 IU penicillin/ml*  Penicillin | 749 *S. aureus* | No statistically significant differences were observed in the prevalence of penicillin resistance in *S. aureus,* or the proportion of *S. aureus* isolates resistant to penicillin between herd groups (ORG, CON, transition year 1, transition year 2, transition year 3). |
| Roesch et al., 2006; Switzerland (EU)  *S. aureus*, NAS | Cross-sectional (1 visit/herd); 5-13 lactating cows (dep. on farm size) randomly selected at 31 DIM (median); Quartermilk samples collected from quarters with CMT ≥ 2+; Isolates from subclinical mastitis | 60 ORG herds, 60 CON herds; ORG herds chosen randomly from interested pool; Matching CON herds selected based on geographic proximity, same agricultural zone (elevation), and farm size  ORG herds certified ≥ 3 yr. | No quantification of AM usage provided, but prophylactic use of AM lower for ORG herds than CON herds  Main AM used for DCT for ORG and CON herds were penicillin (40 and 66%, respectively), cloxacillin (36.5 and 37%, respectively), neomycin (23.5 and 52.7%, respectively), and gentamicin (11.8 and 2.4%, respectively) | *Broth microdilution (custom plates; Sensititre)*  Amoxicillin-clavulanic acid, ceftiofur, chloramphenicol, clindamycin, enrofloxacin, erythromycin, gentamicin, oxacillin, quinupristin-dalfopristin, penicillin, tetracycline, vancomycin | 79 *S. aureus*: 33 CON, 46 ORG  38 NAS: 19 CON, 19 ORG | Percentage of antibiotic resistance did not differ significantly between *S. aureus* and NAS isolates from cows kept on ORG and CON herds for 12 antimicrobials representing either drugs used to treat mastitis in dairy herds, or drugs important in human medicine. The proportion of resistant *S. aureus* isolates was numerically higher from ORG cows (16/46, 35%) vs. CON cows (6/33, 18%), but this difference was not statistically significant. The proportion of resistant CNS isolates was very similar from ORG cows (9/19, 47%) and CON cows (10/19, 53%). NAS isolates had a higher percentage of antibiotic resistance than *S. aureus* isolates. |
| Bombyk et al., 2007; US  Coagulase-positive *Staph.* (CPS), Novobiocin-sensitive CNS (NSCNS), Novobiocin-resistant CNS (NRCNS) | Cross-sectional (1 visit/herd); Composite quartermilk samples collected from "all healthy cows;" Not specified if isolates from clinical or subclinical mastitis | 8 ORG herds, 8 CON herds; All small dairies (20-100 cows), herds not matched  ORG herds certified ≥ 1 year under USDA National Organic Program (no AM usage for ≥ 4 yr.: 1 yr. certified, 3 yr. of transition) | No quantification of AM usage provided  CON herds reported usage of several AM drugs in the past year: cephalosporins (7 herds), penicillins (6 herds), tetracyclines (5 herds) and pirlimycin (5 herds), and 5 herds practiced blanket DCT | *Disk diffusion*  Cefoxitin, cephalothin, erythromycin, novobiocin, penicillin, pirlimycin, tetracycline, vancomycin | 36 *S. aureus*: 9 CON, 27 ORG  210 NSCNS: 55 CON, 155 ORG  159 NRCNS: 102 CON, 57 ORG | Organic dairy management was associated with more overall antimicrobial susceptibility among staphylococci than was conventional management. In an analysis combining all (3) groupings of staphylococci, a larger proportion of isolates from ORG herds were susceptible to pirlimycin and tetracycline compared with those from CON herds. Susceptibility to erythromycin and penicillin did not differ significantly by herd type when all staphylococci were combined (CON vs. ORG).  When broken down by category of CNS (novobiocin susceptible or resistant), isolates within both CNS categories from ORG herds were more likely to be susceptible to pirlimycin than CNS from CON dairies. No difference in tetracycline, erythromycin or penicillin susceptibility was seen between herd types (CON vs. ORG) within either CNS category. A larger proportion of NSCNS vs. NRCNS for both CON and ORG herds were susceptible to tetracycline, leading the authors to suggest that management practices unrelated to antimicrobial use may contribute to the observed differences in susceptibility patterns of CNS on dairy herds. |
| Pol and Ruegg, 2007; US  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Quartermilk samples from a maximum of 50 multiparous cows with no signs of clinical mastitis; Multiparous cows sampled to ensure at least 1 known exposure to intramammary antimicrobial drugs (DCT); Isolates from subclinical mastitis | Herds categorized based on amount of antimicrobial exposure: 20 ORG herds (no usage); 15 conventional–low usage herds (CON-LO) herds not using or using less than or equal to the first quartile of use of each AM compound); 5 conventional–high usage herds (CON-HI) herds using more than the first quartile of a particular AM compound); All herds had 6-mo. avg. bulk tank SCC ≥250,000 cells/mL; CON herds required to have used blanket DCT for at least 5 yr.; Herds not matched  ORG herds certified ≥ 3 yr. | AM usage quantified at both herd and cow level as defined daily dose (DDD).4 Herd-level DDD was calculated by dividing the reported total dose of each drug used per year by the DDD of that AM. Number of DDD was divided by the total number of milking cows to estimate the density of use of particular AM (expressed as number of DDD per lactating cow per year)  β-Lactams, including cephapirin, penicillin, and ceftiofur, were used on the majority of the herds. Cephapirin and penicillin were used as intramammary infusions (treatment of clinical mastitis, DCT). Detailed description of AM usage by drug provided in reference | *Broth microdilution (Mastitis panel; Sensititre)*  Ampicillin, ceftiofur, cephalothin, erythromycin, oxacillin + 2% NaCl, penicillin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline | 137 *S. aureus*: 52 CON (15 herds), 85 ORG (18 herds); Range of no. isolates used per herd: CON: 1-9, ORG 1-18  295 CNS: 160 CON (20 herds), 135 ORG (19 herds); Range of no. isolates used per herd: CON: 2-16, ORG 1-16 | Authors took multiple approaches to compare resistance among isolates from the 3 antimicrobial usage groups:   1. Compared proportion for each type of isolate (CNS or *S. aureus*) that was susceptible or resistant in each category (CON vs. ORG) using χ2 test of association, in order to ask if proportion of susceptible isolates independent of herd type 2. Used χ2 test to explore if the MIC for each type of isolate (CNS or *S. aureus*) was independent of herd type (CON vs. ORG) 3. Performed survival analysis of each type of isolates (CNS or *S. aureus*) based on the 3 antimicrobial usage categories (ORG, CON-LO, or CON-HI). Antimicrobial concentrations in wells of the susceptibility test were used as “time,” and event was inhibition of bacterial growth   In order to avoid statistical dependence, only 1 isolate per cow and no more than 20 isolates per herd were included in the analysis. Overall, isolates from ORG herds were more susceptible to antimicrobials than those from CON herds. The authors stress that although some differences were found between antimicrobial groups, most isolates of both types were inhibited at the lowest dilution tested of most antimicrobial drugs.  *S. aureus:*   1. *S. aureus* isolates from CON herds were more likely to be resistant to ampicillin and penicillin compared with isolates from ORG herds. Herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested 2. *S. aureus* isolates from CON herds had a higher MIC for pirlimycin and sulfadimethoxine compared with isolates from ORG herds. Herd type was not associated with the MIC of the other antimicrobial drugs tested 3. In the survival analysis, the MIC that inhibited 90% (MIC90) of *S. aureus* isolates from ORG herds for penicillin and pirlimycin was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (MIC50, the MIC that inhibited 50% of isolates, was not different for these drugs)   *CNS:*   1. CNS isolates from CON herds were more likely to be resistant to ampicillin, penicillin, pirlimycin, and tetracycline compared with isolates from ORG herds. Herd type was not associated with the proportion of resistant isolates for the other antimicrobial drugs tested 2. CNS isolates from CON herds had a higher MIC for ampicillin, pirlimycin, and tetracycline compared with isolates from ORG herds. Herd type was not associated with the MIC of the other antimicrobial drugs tested 3. In the survival curve analysis, the MIC that inhibited 90% (MIC90) of CNS isolates from ORG herds for ampicillin, penicillin, pirlimycin, and tetracycline was lower than the MIC90 of the isolates from CON-LO and CON-HI herds (ORG and CON-LO herds had a lower MIC50 for erythromycin than CON-HI herds, but the MIC90 did not differ by usage group) |
| Garmo et al., 2010; Norway (EU)  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Quartermilk samples from all lactating cows; Isolates from subclinical mastitis | 25 CON herds, 24 ORG herds; All herds Norwegian Red cows; Matching CON herds selected based on herd size (± five cow-years) and type of housing  ORG herds certified ≥ 4 yr. | No quantification of AM usage provided  Generally, Benzyl penicillin and dihydrostreptomycin are the most common antimicrobials used for intramammary treatment in Norway | *Cloverleaf lactamase test*  Penicillin | 132 *S. aureus*: 68 CON, 64 ORG  260 CNS: 167 CON, 93 ORG | Proportions of *S. aureus* and CNS isolates from ORG herds resistant to penicillin were similar to those from CON herds, although no statistical comparison was carried out. Penicillin resistance was proportionately higher in CNS vs. *S. aureus* isolates*.*  *S. aureus:*  6 out of 68 (8.8%) isolates from CON herds were penicillin-resistant, compared with 9 out of 64 (14.0%) from ORG herds.  CNS:  81 out of 167 (48.5%) isolates from CON herds were penicillin-resistant, compared with 93 out of 200 (46.5%) from ORG herds. |
| Cicconi-Hogan et al., 2014; US  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Bulk tank milk | 192 ORG herds, 100 CON herds; Matching CON herds selected based on proximity to ORG herd and herd size category (0–99, 100–199, or ≥200 adult cows)  No. yr. ORG herds certified not provided | No quantification or description of AM usage provided | *Detection of mecA gene by PCR, MRSASelect plates (Bio-Rad Laboratories Inc.)*  β-lactamase resistance (MRSA*Select* plates used to screen for methicillin resistance, and contain a proprietary combination of an unspecified β-lactam, lithium chloride, aztreonam and cycloheximide) | Not provided | 13 isolates from bulk tank milk were identified as methicillin resistant (positive for mecA gene): 7 from CON herds, 6 from ORG. Species identification of isolates from bulk tank milk was performed using 16S rRNA and rpoB genes.  These 13 isolates were identified as *S. aureus* (n = 1), *S. sciuri* (n = 5), *S. chromogenes* (n = 2), *S. saprophyticus* (n = 3), *S. agnetis* (n = 1), and *Macrococcus caseolyticus* (n = 1). The single methicillin-resistant *S. aureus* isolate was from an ORG herd, for an observed 0.3% prevalence at the herd level. The methicillin-resistant CNS prevalence was 2% in the organic population, and 5% in the conventional population.  The authors highlight the high number of methicillin-resistant *S. sciuri* identified (6 out of 12 methicillin resistant CNS) compared to previous work, and also suggest that a potential methicillin-resistant *Staphylococcus* reservoir in the dairy herd population of the United States may be independent of production system type (CON vs. ORG). |
| Tenhagen et al., 2018; Germany (EU)  *S. aureus* | Cross-sectional (1 visit/herd); Bulk tank milk | 372 CON herds, 303 ORG herds; Minimum herd size 30 lactating cows; Selection of herds based on sampling plan designed to cover German states according to their share of national CON and ORG cow population; Separate sampling plans for the 2 categories as proportion ORG herds comparatively low  No. yr. ORG herds certified not provided | No quantification or description of AM usage provided | *Broth microdilution*  Cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, penicillin, quinupristin/dalfopristin, rifampicin, sulfamethoxazole, streptomycin, tetracycline, tiamulin, trimethoprim, vancomycin | Not provided | Genomic methods used for identifying isolates to the species level (multiplex PCR: 23S rDNA, specific for Staph; *nuc* gene, specific for *S. aureus*; *mecA* gene, β-lactam resistance)  Used a binary logistic regression to describe association of methicillin-resistant *S. aureus*-positive samples with herd type (CON vs. ORG), controlling for effect of region and herd size (both significant predictors of MRSA herd status)  The prevalence of MRSA was significantly higher in BTM samples from CON herds (9.7%) compared with ORG herds (1.7%). Proportion of methicillin-resistant *S. aureus* isolates resistant to 12 different antimicrobials tended to be higher from bulk tank milk samples of CON herds (vs. ORG herds). As there were limited number of isolates from ORG herds (n = 5) compared to CON herds (n = 36), no statistical tests were performed |
| McDougall et al., 2020; New Zealand (US organic regulations)  *S. aureus*, CNS | Cross-sectional (1 visit/herd); Quartermilk samples from cows that had had at least 1 lactation, had been treated with DCT (in herds using DCT), had not been treated with any other antimicrobial within 30 d before sample collection, and had an individual SCC of >200,000 cells/mL; Not specified if isolates from clinical or subclinical mastitis | 7 ORG herds, 11 CON herds using ampicillin-cloxacillin DCT (CON-AC), 8 CON herds using cephalonium DCT (CON-CE); CON herds selected on the basis that >50% of cows were treated in each of the 3 previous yr. with 1 DCT product; Herds not matched  ORG herds certified ≥ 3 yr. (median = 12 yr.; range = 7-19 yr.) | Herd-level use of antimicrobials estimated by extracting AM sales data for each herd for the previous 3 yr. to determine total mass of antimicrobials used per kilogram of liveweight per year for each herd, and mass of each class of AM per kg of liveweight per year  β-lactam AM most commonly used DCT products in New Zealand generally, with 25% containing ampicillin, 61% containing cloxacillin, and 13% containing cephalonium, by mass | *Broth microdilution (Mastitis CMV1AMAF; Thermo Scientific)*  Ampicillin, ceftiofur, cephalothin, erythromycin, oxacillin, penicillin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, tetracycline | 320 *S. aureus*: 111 CON-CE, 99 CON-CA, 110 ORG  240 CNS: 82 CON-CE, 74 CON-CA, 84 ORG | Overall, the authors found that the MIC of CNS from ORG herds were lower than isolates from both types of CON herd. However, they point out that these differences in MIC occurred below clinical breakpoints, and therefore may not affect bacteriological cure rates. They found bimodal distributions of MIC for ampicillin and penicillin in *S. aureus* isolates from ORG herds, and suggest either (1) isolates with a higher MIC are “a natural part of the bacterial population of the bovine mammary gland,” or (2) isolates with higher MIC have persisted within ORG herds since antimicrobial usage was occurring on the farm  *S. aureus:*  The MIC50 for ampicillin and penicillin were greater bymorethan1dilutionfor *S. aureus* isolates from CON-CE herds compared with CON-CA and ORG herds, but this relationship did not hold for the MIC90 of these drugs (MIC for CON-CE and ORG herds greater than CON-CA).  In a univariate analysis, the proportion of penicillin-resistant *S. aureus* isolates was significantly higher in CON-CE herds (76/111; 68.5%) compared to CON-CA(4/99;4.0%)orORG herds (32/110; 29.1%). A multilevel model (accounting for clustering of quarter within cow within herd) was made where the 3 herd types were the main explanatory variable. Other potential variables offered to this model included age, breed, DIM, SCC, and antimicrobial treatment history for that cow.  In the multilevelmodel,proportionsofpenicillin-resistantisolatesdidnotdifferbetweenisolates from the 3 herd types.  When comparing proportion of *S. aureus* isolates falling into 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were fewer ORG isolatesin the middle category (1 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.  When comparing proportion of *S. aureus* isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance, the only significant difference was that there were more ORG isolatesin the lowest category (32 μg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.  There were no significant differences between the 3 herd types when comparing the proportion of *S. aureus* isolates falling into 3 different breakpoint groups for erythromycin resistance.  CNS:  The MIC50 and MIC90 for ampicillin and penicillin were lower by more than 1 dilution for CNS isolates from ORG herds compared to both types of CON herds; otherwise, these values did not differ by more than 1 dilution between the 3 herd types for the other antimicrobials tested.  In a univariate analysis, proportions of penicillin-resistant CNS isolates were significantly greater in both types of CON herds (CON-CE, 42/82; 51%; CON-CA, 22/74; 30%) than ORG herds (14/84; 17%). Similar to the analyses for *S. aureus,* a multilevel model was also made to compare penicillin resistance with herd type as the main explanatory variable. In this multilevel model, proportion of penicillin-resistant CNS isolates was significantly greater for CON-CE herds (0.50 ± 0.07) compared to CON-CA (0.31 ± 0.06) or ORG herds (0.17 ± 0.05).  When comparing proportion of CNS isolates falling into 3 different breakpoint groups for ceftiofur resistance, the only significant difference was that there were more ORG isolates in the lowest (0.5 μg/mL) and highest (2 μg/mL) categories compared to both CON herd types; otherwise, there were no differences in the proportion of isolates falling into the different breakpoint groups from each of the 3 herd types.  There were no significant differences between the 3 herd types when comparing the proportion of CNS isolates falling into 3 different breakpoint groups for sulfadimethoxine resistance.  When comparing proportion of CNS isolates falling into 3 different breakpoint groups for erythromycin resistance, the only significant difference was that there were more CON-CA isolates in the highest category (≥1 mg/mL); otherwise, there were no differences in the proportion of isolates falling into the different breakpoints from each of the 3 herd types. |
| 1 Terminology used is consistent with authors’ language and groupings of organisms (e.g., NAS vs. CNS) | | | | | | |
| 2 Manufacturer information provided when specified | | | | | | |
| 3 Unpublished survey on antibiotic resistance performed in Swiss dairy farms by the Swiss Federal Dairy Research Station (Schallibaum, 1992) | | | | | | |
| 4DDD is the maximum dose a standard animal (BW = 680 kg) would receive if it were treated following the FDA-approved label dosages | | | | | | |

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| Table 2. Observational studies describing species-specific antimicrobial susceptibility of staphylococci isolates from bovine intramammary infections. Ten studies are included which describe phenotypic resistance profiles and isolates were speciated using genotypic techniques or MALDI-TOF. NAS= non-*aureus* staphylococci; CNS = coagulase-negative staphylococci; AMR = antimicrobial resistance; CM = clinical mastitis; SCM = subclinical mastitis | | | |
| *Reference*  *Country* | *Number of isolates1*  *CM or SCM associated* | *Methodology* | *Overall findings* |
| Sampimon et al., 2009  The Netherlands | 170 CNS  Not specified | Broth microdilution; PCR for *blaZ, mecA, ermA, ermB, ermC, msrA, lnuA, msrA, mphC* | Significant differences in resistance patterns were found between CNS species. Phenotypic resistance and resistance genes were relatively rare in *S. chromogenes*, with the exception of *blaZ* (which was present in 80% of all CNS isolates).  For phenotypic resistance, *S. fleuretti* and *S. epidermidis* had the highest resistance to penicillin, oxacillin resistance was most commonly found in *S. fleurettii, S. cohnii, and S. xylosus*, and resistance to macrolide antibiotics was most prevalent in *S. cohnii*, *S. equorum*, and *S. epidermidis.* There was a high prevalence of genotypic resistance (particularly *mecA*) or presence of multiple resistance genes in species with relatively a low prevalence (*S. cohnii, S. equorum, S. fleurettii, S. sciuri*).  The authors note that the resistance profile of *S. epidermidis* was of the most concern; it was the second most commonly found species, carried multiple resistance genes in ~50% of isolates, and phenotypic penicillin resistance was more common compared to other CNS. |
| Persson Waller et al., 2011  Sweden | 154 CNS  Compares clinical and subclinical | Broth microdilution; Cloverleaf β-lactamase test | Overall, prevalence of antimicrobial resistance for CNS was low, but some variation between species was observed. β-Lactamase production was the most common resistance mechanism found, with 29% of isolates found to be positive. The prevalence isolates of producing β-lactamase varied markedly between species. β-lactamase production was significantly higher for *S. epidermidis* and *S. haemolyticus* (40%) compared to *S. simulans* and *S. chromogenes*, where none or only a few of the isolates were β-lactamase positive. Resistance to other antimicrobials besides penicillin was uncommon, and was markedly lower than previous work describing erythromycin, oxacillin and tetracycline resistance levels in CNS. |
| Frey et al., 2013  Switzerland | 408 CNS  Compares clinical and subclinical | Broth microdilution; PCR for *mecA, mecC* | Overall phenotypic resistance: oxacillin resistance (indicator of *mec* gene-mediated methicillin resistance) was the most frequently identified (47.0% of all isolates), and was more frequent in clinical (56.5%) vs. subclinical mastitis isolates (43.9%). In order, the next most common resistances to antimicrobials identified were fusidic acid (33.8% of isolates resistant), tiamulin (31.9%), penicillin (23.3%), tetracycline (15.8%), streptomycin (9.6%), erythromycin (7.0%), sulfonamides (5%), trimethoprim (4.3%), clindamycin (3.4%), kanamycin (2.4%), and gentamicin (2.4%)  Resistance to oxacillin was attributed to *mecA* gene in 9.7% of oxacillin-resistant isolates, while remaining oxacillin-resistant CNS did not contain *mecC* or *mecA1* promoter mutations. Isolates of *S. fleurettii, S. epidermidis, S. haemolyticus,* and *S. xylosus* were identified as carrying the *mecA* gene. Resistance to tetracycline was attributed to the presence of *tetK* and *tetL* genes, penicillin resistance to *blaZ*, streptomycin resistance to *str* and *ant(6)-Ia*, and erythromycin resistance to *ermC, ermB,* and *msr* genes. |
| Taponen et al., 2016  Finland | 400 CNS  Combines clinical and subclinical | Broth microdilution | *S. simulans, S. chromogenes, S. haemolyticus,* and *S. epidermidis* differed in their antimicrobial susceptibility, with penicillin resistance was the most common type of antimicrobial resistance identified. Phenotypic oxacillin resistance was found in all four species (34% of the isolates overall). Whereas the majority of *S. epidermidis* isolates were resistant to benzylpenicillin, only a few *S. simulans* isolates were penicillin-resistant. 21 isolates (5% of isolates overall) were positive for the *mecA* gene (20 *S. epidermidis*, 1 *S. sciuri*).  *S. epidermidis* was the most resistant among the four major species studied, as resistance to antimicrobials was common, several isolates were multidrug resistant, and 19% of isolates were *mecA*-positive (encoding methicillin resistance). |
| Raspanti et al., 2016  Argentina | 219 CNS  Not specified | Broth microdilution | Overall, 51.6% of isolates were resistant to penicillin. The MIC90 value for penicillin was > 8g/ml for CNS isolates included in the study, which the authors note was well above the recommended breakpoint. Fourteen percent of all CNS isolates tested were resistant to oxacillin (of which 16.7% were *mecA* positive), 29.2% to erythromycin and 30.1% to tetracycline. *S. chromogenes* and *S. haemolyticus* showed a very high proportion of isolates resistant to penicillin (45.1% and 58.6%, respectively).The proportion of penicillin-resistant isolates was smaller for *S. warneri* (4/16), and no resistance to oxacillin was observed. In *S. xylosus,* penicillin resistance was the most common among the species tested (13/14 isolates). |
| Mahato et al., 2017  India | 62 CNS  Clinical isolates | Disk diffusion; PCR for *mecA, mecC, vanA* | As a whole, CNS demonstrated a high level of resistance toward oxacillin (85.5% of isolates) and cefoxitin (83.9%), moderate resistance against rifampicin (37.1%), clindamycin (32.3%), erythromycin (25.8%), and tetracycline (20.9%), and a low level of resistance against ciprofloxacin (11.3%) and gentamycin (9.7%). All strains were susceptible to vancomycin, teicoplanin and linezolid. The methicillin resistance gene *mecA* was found in 95.16% of isolates. *S. sciuri* and *S. haemolyticus* had the highest proportion of methicillin resistant isolates. |
| Nobrega et al., 2018  Canada | 1,702 NAS  Combines clinical and subclinical | Broth microdilution (1,702 isolates); whole genome sequencing (405 isolates) | Prevalence of resistance to important antimicrobials highly important frequently used in dairy herds was relatively common (β-lactams: 10%, tetracyclines: 10%), as was resistance to erythromycin (6%), but resistance to antimicrobials critically important for human medicine (vancomycin, fluoroquinolones, linezolid and daptomycin) was rare (<1%). The most frequently identified genetic resistance determinants were mutations in the *folP* gene and MDR efflux pumps; these mutations were present in all NAS isolates and not associated with a multi-drug resistant phenotype. For NAS species intrinsically resistant to novobiocin, specific residues were found in the in *gyrB* gene. The authors were able to link the presence of *blaZ, mecA, fexA*, *erm, mphC, msrA,* and *tet* genes with drug-specific resistance.  In this study, phenotypic antimicrobial resistance patterns were “clearly species-dependent.” Resistance to quinupristin/dalfopristin was common in *S. gallinarum* (98% prevalence), and *S. cohnii* and *S. arlettae* were frequently resistant to erythromycin (prevalence of 63 and 100%, respectively). The authors highlight *S. arlettae* as particularly concerning in its AMR profile; it had the highest prevalence of AMR against penicillin (61%), ampicillin (23%), erythromycin (100%), pirlimycin (18%) and clindamycin (99.9%), as well as the highest prevalence of MDR. Species-specific patterns were also seen in the prevalence of some AMR genetic determinants. *mecA* elements had a 17% prevalence in *S. epidermidis*, but were close to zero for other species. *erm* genes (encoding rRNA adenine N-6- methyltransferases) were found only in *S. epidermidis, S. cohnii, S. equorum*, and *S. chromogenes*. |
| Fergestad et al., 2021  Belgium and Norway | 227 NAS, 45 *S. aureus*  Combines clinical and subclinical | Disk diffusion; PCR for *mecA, mecC* | Staphylococci isolates were analyzed as 3 separate collections from previous studies (1 in Norway, 2 from different regions of Belgium). Over all 3 sample groups, descriptive analyses showed that antimicrobial resistance was more widespread in several NAS species when compared with *S. aureus* isolates (not including MRSA). Resistance to penicillin was most frequently identified in the Norwegian isolate group. Regardless of sample group, AMR was frequently observed in *S. epidermidis* and *S. haemolyticus*. Resistance to trimethoprim-sulfonamide was frequently observed in *S. aureus,* *S. epidermidis*, and *S. haemolyticus*. |
| Taponen et al., 2023  Finland | 244 NAS, 260 *S. aureus*  Not specified | Disk diffusion; PCR for *mecA, mecC, blaZ* | Authors found that penicillin resistance was the only significant form of AMR from staphylococci associated with IMI in Finland, with 18.8% of all isolates (*S. aureus*: 9.3%; NAS: 28.9%) found to be resistant by disk diffusion. Genotypic potential for resistance to β-lactamases was higher, with *blaZ* found in 26.6% of all isolates (*S. aureus*: 18.5%; NAS: 35.2%). In a phenotypic test detecting production of β-lactamases (nitrocefin test), 21.5% of all isolates were positive (*S. aureus:* 11.6%; NAS: 32.0%). Species-specific differences were observed in penicillin resistance, with the proportion of penicillin-resistant being lowest in *S. simulans* and highest in *S. epidermidis*, and *S. epidermidis* accounting for 6/8 NAS isolates carrying the *mecA* gene. |
| Yang et al., 2023  China | 160 CNS, 172 *S. aureus*  Clinical isolates | Disk diffusion; PCR for *blaZ, mecA, mecC, tetK, tetM, ermA, ermB, ermC* | Overall, both phenotypic and genotypic resistance was highest amongst *S. aureus* and CNS for penicillin, followed by erythromycin and tetracycline. Phenotypically, *S. aureus* isolates showed the highest resistance rates to penicillin (58.7%), followed by erythromycin (22.1%), tetracycline (15.1%), gentamicin (10.5%), ciprofloxacin (8.7%), and chloramphenicol (5.8%). CNS isolates displayed high phenotypic resistance to penicillin (71.3%), followed by erythromycin (28.8%), tetracycline (19.4%), gentamicin (9.4%), chloramphenicol (7.9%), ciprofloxacin (2.5%), and cefoxitin (1.3%).  *blaZ* was detected in 61.0% of *S. aureus* isolates, with all penicillin-resistant S. aureus isolates positive for the gene. *tetK* and *tetM* were found in 12.2% and 9.9% of *S. aureus* isolates, respectively, with all *tetK/tetM*-positive isolates showing resistance to tetracycline. *ermC* and *ermB* were found in 22.1% and 13.4% of *S. aureus* isolates, respectively, with all erythromycin-resistant isolates carrying ermC alone or in combination with *ermB*. No *S. aureus* were positive for *mecA, mecC or ermA.* For CNS isolates evaluated, *blaZ* was found in 69.4% isolates with all showing resistance to penicillin. One each *S. equorum* and *S. saprophyticus* that were resistant against penicillin were negative for blaZ but carried *mecA*. *tetK* and *tetM* were found in 17.5% and 12.5% CNS isolates, respectively, with all *tetK*/ *tetM*-positive isolates showing resistance to tetracycline. *ermC* and *ermB* were found in 28.1% and 16.9% of CNS isolates, respectively, with all erythromycin-resistant isolates carrying *ermC* alone or in combination with *ermB*. No CNS were positive for *mecC* or *ermA*. |
| 1 Terminology used is consistent with authors’ language and groupings of organisms (e.g., NAS vs. CNS) | | | |

Figure 1.Adapted from Call et. al, 2008. A proposed model illustrating how antimicrobial resistance can be maintained in a farm environment despite the absence of antimicrobial selection pressure, primarily based on studies of resistant bacteria in the GI tract of cattle. Antimicrobial treatment of an individual animal leads to a transient expansion of AMR subpopulations within the gut, as resistant bacteria have a selective advantage. Eventually, the antimicrobial-induced expansion of the resistant population abates when the selective force of antimicrobial use is removed. If there is a fitness cost for maintenance of AMR for an organism, the relative proportion of AMR subpopulations decline in the absence of antimicrobials. However, expansion of the resistant population also increases the likelihood of a genetic event where an AMR gene is linked to another trait, one that confers a niche-specific fitness advantage to the resistant bacteria. If this selective linkage of AMR occurs, maintenance of a baseline prevalence of the AMR subpopulation may occur, despite the lack of selective pressure from antimicrobial use.