***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, it is generally accepted that any use of antimicrobial agents is a “powerful selective force that promotes the emergence of resistant strains,” and that 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, which is often another species of bacteria (Chambers, 2001; Sefton, 2002). When horizontal transfer occurs, antimicrobial resistance genes can become rapidly and widely disseminated throughout a population either by further genetic exchanges between the newly-resistant strain and other susceptible strains, or by clonal spread of the newly-resistant strain itself (Chambers, 2001).Although the associations between bacterial susceptibility, antimicrobial use, and resistance 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). Further, 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 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 resistant bacteria from farm systems to humans is not fully understood, as selection for resistant bacteria and transfer of antibiotic resistance genes occurs through a variety of mechanisms and is not always linked to use of a specific antibiotic (Mathew et al., 2007).

According to Call et al. (2008), the most “obvious selection pressure for AMR” on cattle farms is the use of antimicrobials for treating sick animals, which can promote AMR by two potential mechanisms: 1) treatment with antimicrobials provides a competitive advantage for strains that carry resistance to that particular drug, allowing resistant bacterial populations to increase; and 2) if resistance genes are harbored on horizontally transmissible elements (plasmids, conjugative transposons), strains carrying these elements can then successfully disseminate them to new, previously-susceptible bacteria. 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 in dairy animals globally (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 types of 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 intramammary infections (Kolar et al., 2024; Pol and Ruegg, 2007b; with the notable exception of *S. aureus* and NASM against penicillin). However, continued surveillance of AMR patterns for these ubiquitous mastitis pathogens is warranted, as 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). Additionally, studies reporting evidence of transmission of resistance genes between different staphylococcal species support 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).

An interesting way 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 differs between countries in the EU and the US (see below), “organic” dairies overall use little to no antimicrobials on the farm when compared to “conventional’” dairy farms. When comparing bacterial isolates of bovine origin from these two types of systems, the general hypothesis is that antibiotic resistance may likely diminish in prevalence when antibiotic use is decreased or discontinued. When the selective pressure of antimicrobial usage is removed (as on organic dairies), bacterial strains that may contain resistance genes are often gradually replaced by susceptible strains. The goal of this narrative review was to summarize studies which analyzed the relationship between antimicrobial usage at the farm level (organic vs. conventional), and antimicrobial susceptibility of bovine staphylococcal mastitis isolates.

* + Summarized in Call 2008
    - post-hoc analysis of individual studies is highly problematic due to differences in methods used (e.g. disc diffusion versus serial broth dilution and changing criteria) (Klement et al., 2005); failure to speciate the organisms under study when there can be considerable variation between species and strains (Rossitto et al., 2002); changes in management practices; differences in sample collection and culture methods can bias recovery of organisms; differences in sampling frame (independence between isolates; random, opportunistic, or clinical sampling) can also introduce bias; stochastic events (e.g. heterogeneous clonal dissemination) could easily bias interpretation of smaller studies; even well-organized, large-scale, and centralized studies encounter deviations in study protocols and unequal reporting efforts that make comparisons between countries tenuous (Hendriksen et al., 2008).
      * Clinical cases: analyses of clinical isolates, it is important to acknowledge that resistant isolates may be amplified by therapeutic treatments that are administered to sick animals prior to isolation of resistant organisms; this may bias prevalence estimates for AMR pathogens compared with a random sampling design
    - as with all correlation studies readers should be cautious about inferring causation when there are limited controls for confounding variables or when conclusions are drawn from a limited number of independent observations
  + *“variation among herds in MIC may in part be due to introduction of resistant isolates, rather than selection for, or perpetuation of, such isolates within a herd. Additionally, other mastitis management practices may affect the probability that resistant isolates remain in the herd. For example, selection criteria for culling of cows may remove cows infected with resistant isolates”* McDougall 2021

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

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There are many important considerations when comparing results between studies reporting antimicrobial sensitivity of mastitis pathogens on organic and conventional farms. Importantly, methodology used to determine MIC or categorization of an isolate as susceptible, intermediate, or resistant varies between studies (Table XX). Discrepancies exist between phenotypic and genotypic test results, due either to detection of phenotypic resistance in the absence expected genotypic determinants, or phenotypic susceptibility despite presence of genotypic determinants. For bovine mastitis *S. aureus* isolates, both of these types of discrepancies have been reported for penicillin resistance (Sampimon, 2009; Taponen et al., 2023). This also holds true for other staphylococci: “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” (Sampimon, 2009). Taponen et al. (2023) compared different methods of testing for β-lactamase resistance in staphylococci from cases of bovine mastitis. Overall agreement between phenotypic and genotypic resistance tests was moderate to substantial, but they did find some inconsistencies between methods of identifying penicillin resistance between phenotypic susceptibility by disk diffusion method, the nitrocefin test to assess β-lactamase production, and PCR to detect the and the presence of *blaZ, mecA*, and *mecC* genes. Discrepancies also exist between methods of phenotypic determination of resistance for mastitis isolates. A study comparing agreement between the broth microdilution (Sensititre Custom Plates) and the agar disk diffusion method for determining antimicrobial susceptibility of isolates from bovine mastitis found fair agreement overall (80.7%) between the two methods, but this varied based on the particular bacterial-antimicrobial combinations tested (Palladini et al., 2023). No NAS species were tested, 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 antimicrobial resistance profiles of clinical mastitis pathogens, agreement was good between the two methods for most isolate-antimicrobial MIC combinations (Saini et al., 2011). An important caveat in this study was that the diagnostic accuracy was low for *S. aureus*-ceftiofur/oxacillin combinations with either testing method. Low correlation between the two methods was also found for *S. aureus* and erythromycin, and *S. aureus* and neomycin in another study comparing dilution methods to determine MIC and disk diffusion diameters for mastitis bacterial isolates (Klement et al., 2005). Further complicating comparison of AMR between studies is shifting criteria for classifying an isolate as susceptible or resistant, as breakpoints for antimicrobial susceptibility testing are updated every few years, and the existence of multiple conflicting standards for categorization of resistant or susceptible bacteria dependent on geographical location (Clinical & Laboratory Standards Institute, CLSI; European Committee on Antimicrobial Susceptibility Testing, EUCAST).

Difference in sampling scheme of studies collecting milk from cows will affect resistance profiles in bacteria isolated from samples. Within the studies summarized in Table XX, sampling strategy 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 CMT to selectively sample cows in a herd with 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* intramammary infections, while others only sampled multiparous cows in the herd (Pol and Ruegg, 2007a; McDougall et al., 2021). Sampling multiparous cows will increase the proportion of samples collected with an intramammary infection, as increasing parity is a risk factor for mastitis generally (Barkema et al., 1998; Busato et al., 2000) and intramammary infection with *S. aureus* specifically (Zadoks et al., 2001; Tenhagen et al., 2006). Furthermore, prevalence of distribution of NASM species varies by parity (see above/below XX), so sampling multiparous cows exclusively will bias which species are included and thereby the resistance profiles of mastitis pathogens described (as resistance patterns are species-specific for NASM).

An additional consideration of studies describing AMR of isolates from bovine mastitis is whether the bacteria were associated with cases of subclinical mastitis cases, clinical mastitis cases, or both (or not specified; Table XX). AMR is more prevalent in NASM isolates associated with clinical vs. subclinical mastitis, so the type of mastitis sampled included in a study will affect the amount of resistance observed in isolates associated with bovine mastitis. Oxacillin resistance was more frequent in clinical mastitis isolates (56.5%) than in subclinical mastitis isolates (43.9%; Frey et al., 2013), and β-lactamase production was more common in subclinical vs. clinical cases (Persson Waller et al., 2011). Wuytack et al. (2020) found that carriage of the resistance gene *mecA* was proportionately higher in NASM isolates causing clinical vs. subclinical infection. Therefore, inclusion criteria for milk samples associated with either clinical or subclinical mastitis will affect the observed AMR prevalence. However, as there is evidence that certain NASM species are more likely to be associated with cases of clinical mastitis vs. subclinical mastitis and vice versa versa (Persson Waller et al., 2011; although, see Condas et al., 2017b) and resistance patterns of NASM are species-specific, differences in AMR prevalence in samples from clinical vs. subclinical NASM mastitis may result from differences in species between the 2 categories. In Persson Waller et al. (2011), *S. epidermidis* and *S. saprophyticus* were more prevalent in subclinical than in clinical mastitis, while *S. hyicus* was more common in clinical mastitis. The authors state that the higher proportion of penicillin resistance in subclinical vs. clinical isolates was likely due to the high prevalence of *S. epidermidis* and *S. saprophyticus* in subclinical mastitis samples, as these 2 species demonstrated significantly more penicillin resistance than other NASM species. Further support that differences in AMR for NASM isolates associated with clinical vs. subclinical mastitis is a result of species differences and not clinical status is found in Naushad et al. (2018). In their analyses of 328 NASM isolates from subclinical mastitis and 57 isolates from clinical mastitis, they found that within the same species, no significant differences existed in the prevalence of drug-specific AMR or resistance determinants when contrasting the two types of samples.

Perhaps the most important caveat when considering the body of work comparing resistance patterns of mastitis pathogens is that “organic” dairy systems are not the same in the US and Europe, where the majority of these studies have been carried out. Organic regulations for European countries still allow for some antimicrobial use (albeit with extended withdrawal periods and stricter veterinary oversight), while organic regulations in the US mandate that any animal treated with antimicrobials is removed from the herd. The level of on-farm antimicrobial usage (and therefore selective pressure for resistance) therefore differs between European (EU Commission, 2024) and US dairies (USDA, 2024), making comparisons between studies carried out under these varying regulations difficult. Regulations for both organic dairy production certifications have evolved over time (Dimitri and Nehring, 2022; Grodkowski et al., 2023), further adding to the nuance of how organic dairy production is defined in both entities. An additional layer of complexity is that 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 (USDA, 2009). Consequently, these geographic and temporal differences must be kept in mind when reviewing studies comparing organic and conventional dairy systems between the EU and US as they could theoretically affect the type and amount of selective pressure mastitis pathogens experience.

***What other factors DO explain differences in antimicrobial susceptibility?***

* ***Most (all) studies didn’t speciate CNS***
* ***Different NASM species vary in AB susceptibility***

Table XX summarizes work describing the species-specific antimicrobial susceptibility of staphylococci isolates from bovine intramammary infections. The 10 observational studies included describe phenotypic resistance profiles, and are limited to works where isolates were speciated using genotypic techniques or MALDI-TOF. Overall, resistance to β-lactam antibiotics is the predominant type of antimicrobial resistance present in NASM as a group. 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 African (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) and 23% (Fergestad et al., 2021), and 29% (Persson Waller et al., 2011), while a Korean study observed that 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 treatment (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 resistant in Argentina, India, and Canada, respectively (Raspanti et al., 2016; Mahato et al., 2017; Nobrega et al. 2018). The marked geographic variation in resistance patterns may likely be due to differing selective pressure in dairy farm systems around the world; which antimicrobials are most typically used, in what amount, and the various regulation around their usage varies widely from country to country.

Another factor explaining differences in AMR is geographical variation in the distribution of NASM species (SEE above/below; XX). Studies comparing resistance profiles of NASM by species consistently show that antimicrobial resistance 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 NASM species, *S. chromogenes,* in comparison to other species (Sampimon, 2009; Persson Waller et al., 2011). A notable exception is the presence of *blaZ* gene, which was found in 80% of all 170 CNS isolates and 87% of *S. chromogenes* in a Flemish study (Sampimon, 2009). β-lactamase production was found to be significantly lower for *S. chromogenes* compared to *S. epidermidis* and *S. haemolyticus* in Sweden (Persson Waller et al., 2011). Although a smaller-scale study in Argentina found a high proportion of *S. chromogenes* were resistant to penicillin (45.1%), both *S. haemolyticus* and *S. xylosus* had a numerically higher proportion of penicillin resistant isolates (58.6% and 92.9%, respectively; Raspanti et al., 2016). Across a number of studies, authors found that some less-commonly isolated NASM species carried the concerning AMR profiles. Sampimon et al. (2011) found that 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*). In Nobrega et al. (2018), resistance to quinupristin/dalfopristin (a combination of 2 drugs used to treat serious nosocomial infections in humans) 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 also 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. A number of studies also point out 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), 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. The proportion of penicillin-resistant isolates were highest for *S. epidermidis* in a Finnish study, with *S. epidermidis* accounting for 6/8 NAS isolates carrying the *mecA* gene (Taponen et al., 2023). Similarly, β-lactamase production was significantly higher for *S. epidermidis* compared to other species (Persson Waller et al., 2011), and *S. epidermidis* was 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 methicillin resistance).

Even within a given species, AMR carriage has even been linked to certain strain types. For *S. aureus*, carriage of methicillin resistance has been linked to particular clonal complexes both in human medicine (Smith et al., 2021; Garrine et al., 2023) and clusters of *spa* ­type for bovine clinical mastitis isolates (Freu et al., 2022). The link 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.

* ***Different animal factors and mgmt. factors influence what NASM species predominate for a farm, animal***

Numerous risk-factors helping to explain the diversity and prevalence of different NASM species associated with mastitis and bulk tank milk have been identified, including regional, herd and animal-level factors. Different times of year were associated with higher likelihood of intramammary infections 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 have been documented, between 4 regions of Canada (Condas et al., 2017a) and 4 states in the US (Jenkins et al., 2019). Although *S. chromogenes* is the dominant species causing intramammary infections 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). It is difficult to discern whether or not these differences in species diversity are truly a function of geographical variation, or are a result of farms in the same region sharing certain management practices which influence NASM species prevalence and diversity. At the herd level, facility type has been shown to explain some of the diversity of NASM species: for herds using a tiestall barn, prevalence of IMI due to *S. simulans*, *S. xylosus, S. cohnii, S. saprophyticus, S. capitis,* and *S. arlettae* was higher, and prevalence for *S. epidermidis* was lower(Condas et al., 2017a)*.* Herds in Canada using a bedded pack system were at higher relative risk for IMI due to *S. chromogenes* and *S. sciuri* (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 dip compared to herds that did, that 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 speciation 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. well), and Petzer et al. (2022) reported proportionally more IMI due to *S. chromogenes* from herds that were pasture-based compared to those that fed TMR, while *S. haemolyticus* was more prevalent among the TMR herds.

In addition to herd-level and regional differences, cow-level factors determining NASM diversity and prevalence 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 in third-lactation and older cows. These findings are consistent with three other studies reporting *S. chromogenes, S. xylosus,* and *S. simulans* were more commonly found in heifers vs. third-lactation and older cows (De Visscher et al., 2016; Condas et al., 2017a; Nyman et al., 2018). Various NASM species are predominant at different stages within a lactation: Dolder et al. (2017) found that *S. xylosus* was more commonly found in early lactation, while *S. warneri* was found in 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, S. haemolyticus, S. xylosus,* and *S. cohnii* increased during 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, 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, S. sciuri,* which the authors point out 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*).

* ***Therefore, without speciation, can’t fully attribute differences to organic vs. conventional mgmt.; good deal of difference could likely due to species diversity/prevalence***
* ***Unmeasured animal or herd-level mgmt. factors could be confounding results***

frame (**independence between isolates**; random, opportunistic, or clinical sampling) can also introduce bias; stochastic events (e.g. **heterogeneous clonal dissemination**) could easily bias interpretation of smaller studies

Another factor to consider for studies looking at the degree of AMR carriage of mastitis isolates is the effect of a particular herd. Consistent with the behavior of a contagious mastitis pathogen, for any given herd, a particular strain or strains of *S. aureus* will predominant (Lange et al., 1999; Zadoks et al., 2000; Freu et al., 2022). If the dominant strain of *S. aureus* causing intramammary infections in a dairy herd happens to carry a certain AMR determinant, a high proportion of isolates from that herd will likely be phenotypically resistant to that particular antimicrobial: not as a result of environmental pressures and selection, but strictly as a result of phylogeny and the behavior of the pathogen itself. As Call et al. (2008) point out, this effect of a dominant strain type within a herd can lead to issues of non-independence between isolates from a particular farm. This would especially be a problem for smaller-scale studies. Pol and Ruegg (2007) directly address this issue in their study of 40 herds. They state that in order to avoid statistical dependence, only 1 isolate per cow and no more than 20 isolates per herd were included in the analysis; additionally, they report the range of isolates used per herd for the different categories of mastitis pathogens. The interplay between phylogeny and different selection pressures due to management factors on the AMR profiles of mastitis pathogens on dairy farms must be incredibly complex; a study looking at *E. coli* found that on average, phylogenetic groupings were different between organic and conventional dairies, suggesting that there may be differences between lineages of *E. coli* in their ability or likelihood of acquiring resistance genes (Walk et al., 2007). From 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.”

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

Overall, studies comparing antibiotic resistance of staphylococci between herds under organic management and herds managed conventionally find either no difference or more susceptibility for isolates originating on organic farms (Table XX). However, these studies vary widely in their approach to exploring this question, primarily in the 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 proportion of *S. aureus* isolates from organic herds resistant to different antimicrobials was similar to those from conventional herds. Similarly, the proportion of resistant isolates of CNS was comparable between the two systems, with the exception of a numerically higher proportion of isolates resistant to rifamyin from organic herds. A limitation of this study is that the data describing the proportion 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, 2023). In a Swiss study comparing the resistance profiles of NAS and *S. aureus* from quartermilk samples, Roesch et al. (2006) also found that NAS isolates had a higher percentage of antibiotic resistance 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 antibiotic resistance did not differ significantly between *S. aureus* and NAS isolates from cows kept on organic and conventional herds. Although the proportion of resistant *S. aureus* isolates was numerically higher from organic cows (16/46, 35%) vs. conventional cows (6/33, 18%), this difference was not statistically significant. The proportion of resistant CNS isolates between systems was very similar (ORG: 9/19, 47%; CON: 10/19, 53%).

Contrastingly, Bombyk et al. (2008) found that overall, staphylococci isolates causing mastitis on organic dairies were associated with more overall antimicrobial susceptibility than those from conventional farms. For this study, researchers differentiated staphylococci isolates from mastitis 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 bacteria isolates between conventional and organic dairy systems 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 mastitis antimicrobials, 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; the strength of association between the proportion of susceptible and resistant isolates and management category was evaluated, as well as numeric differences in mean zone diameter 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 isolates 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 herd conventional and organic dairies in Denmark.

Two studies looking at bulk tank milk focused on detection of staphylococci carrying genetic determinants conferring penicillin resistance (*mecA* and *mecC* genes), an important consideration for public health globally. In a large study surveilling dairy-associated MRSA in Germany, researchers collected bulk tank milk samples from 372 conventional and 303 organic herds (Tenhagen et al., 2018). Using a 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 both geographical region and herd size, both significant of which were also predictors of MRSA herd status. When comparing the proportion of 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 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 bulk tank milk 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 bulk tank milk as methicillin resistant (*mecA* positive): 7 isolates from conventional herds and 6 from organic. Using 16S rRNA and rpoB genes for speciation, 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). Surprisingly, 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 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. 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 *Staphylococcus 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., the authors point out that this finding demonstrates that cows on organic farms may harbor multidrug-resistant staphylococci despite the limited use of antibiotics 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 and presented. As the basis for many of these studies was to explore if the level of usage of antimicrobial drugs in food animals may select for drug-resistant pathogens (Yan and Gilbert, 2004), 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 that may be occurring for intramammary pathogens. Although all antimicrobial usage is prohibited on US dairy farms, 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 standardize exposure to 10 different antimicrobials by calculating of the number of defined daily doses used per cow, and then categorize the 40 enrolled herds based on their respective antimicrobial exposure. Herds are categorized into 3 groups: organic (no antimicrobial usage), conventional–low usage (CON-LO; conventional farms not using or using ≤ to the first quartile of use of each drug), and conventional–high usage (conventional farms using more than the first quartile of a particular drug; CON-HI). Authors took multiple approaches to compare resistance among isolates from the 3 antimicrobial usage groups. First, they compared the proportion for 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 of 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, antimicrobial concentration in wells of the susceptibility test were used as “time,” and the “event” was inhibition of bacterial growth. In order to avoid correlation between the effects of cow, herd, and exposure category, the authors included only 1 isolate per cow and ≤ 20 isolates per herd in all analyses. 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 MIC that inhibited 90% (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 exploring the difference in 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 DCT (CON-AC), 8 conventional herds using cephalonium DCT (CON-CE) in New Zealand (McDougall et al., 2021). Although the study was carried out in New Zealand, 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 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 authors found that 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.In this multilevel model, 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 cut-points were made for each compound.When comparing proportion of *S. aureus* isolates falling into the 3 different cut-point groups for ceftiofur resistance, the only significant difference was that there were fewer organic isolates in the middle cut-point category (1 μg/mL); otherwise, there were no significant differences in the proportion of isolates falling into the different cut-point groups from each of the 3 herd types.When comparing proportion of *S. aureus* isolates falling into 3 different cut-point 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 significant differences in the proportion of isolates falling into the different cut-points 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 cut-point groups for erythromycin resistance. For CNS isolates, they found that 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, proportions of penicillin-resistant CNS isolates were significantly greater in both types of conventional herds (CON-CE, 42/82; 51%; CON-CA, 22/74; 30%) than organic 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 cut-point 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 significant differences in the proportion of isolates falling into the different cut-points 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 cut-point groups for sulfadimethoxine resistance.When comparing proportion of CNS isolates falling into 3 different cut-point 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 significant differences in the proportion of isolates falling into the different cut-points 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, and 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, 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 organic herds from a time when antimicrobials were used on the farm.

Dairy farms in the process of transitioning from conventional management to organic certification provide a unique opportunity to study patterns resistance in an environment of shifting exposure to antimicrobials. In addition to comparing certified and organic farms, Bennedsgaard et al. (2006) also 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, where quartermilk samples were collected from 30 cows at each farm at high risk of infection with *S. aureus* (determined by a score based on a history of high SCC, breed, and lactation). Herds in the “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 the “old organic” herds, but no other significant differences existed between “old organic” herds or the conventional herds and any of the transition groups (transition year 1, transition year 2, transition year 3) with respect to usage of antimicrobial mastitis treatment. 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 transition groups. As the same 19 herds were sampled repeatedly over 3 years, 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 finding that antimicrobial usage also was not significantly different. In contrast, Park et al. (2012) found that β-lactam resistance rates of CNS decreased with the discontinuation of the use 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 received an intramammary product with pirlimycin, and a product with cephapirin, streptomycin and penicillin, or novobiocin and penicillin at dry-off. There was a significant increase in zone diameter for mastitis-associated CNS isolates for cephalothin, cloxacillin, and penicillin when comparing the conventional vs. organic phase. There was no significant change in the 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 antimicrobial resistance 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 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.

* **Why is AMR maintained in organic systems at all?**
  + Call 2008: “*transient expansion of resistant populations can lead to genetic linkage with other selective traits that permit long-term persistence of AMR subpopulations in production environments”*
    - Example of persistence, chloramphenicol banned but still finding resistance 20 years later:
      * One study found that bacteria from retail ground beef from conventional operations had a higher prevalence of chloramphenicol and ceftiofur resistant bacteria, but there were no differences for nine other antimicrobials (LeJeune and Christie, 2004). It should be noted that chloramphenicol has been banned from use in US food animals since 1986 because of the risk of aplastic anemia and elevated risk of lymphoma in humans (Settepani, 1984), and thus the mechanism allowing persistence of chloramphenicol resistance in fecal bacteria is unclear for US cattle populations
  + Lots of other notes about this in Call above
    - Heuristic model they propose
* **Overall significance of this work?**
  + In addition to public health concerns, it is in the best interest of livestock veterinarians keep cows healthy, decrease suffering, make good quality milk in high volumes -- Want to make sure antimicrobials still work to relieve suffering of animals, cure infections and keep being able to provide large volume of high quality fluid milk
  + Call 2008: on dairy farms the majority of antibiotics are used to treat mastitis and yet AMR remains relatively low in mastitis pathogens
  + Many MIC below clinical breakpoints – so, technically still susceptible – so, what is clinical significance? Not really sure. BUT keeping an eye on it; and reporting MIC numbers, not just lumping in as SIR bc those cut points change over time
    - bacteriological cure rates may not differ between isolates of differing MIC
    - kolar 2024- MIC values varied among pathogens forceftiofur, cephalothin, erythromycin, penicillin, pirlimycin, and tetracycline. However, nearly allisolates were susceptible to ceftiofur and cephalothin, indicating that pathogen differences in MIC arenot likely clinically relevant, as these are the two most commonly administered mastitis treatments inthe United States. While differencesin vitrosusceptibility were observed for some antimicrobials,susceptibility was high to cephalosporin-based IMM treatments that are most commonly used anddid not vary among pathogens
  + future directions?
    - Longer term studies for farms transitioning?
    - Speciation and strain typing, with availability of MALDI
    - Exploration into why AMR stays in ecosystem

**Which AB’s chosen** – relevance to human medicine or most common drugs used for mastitis therapy

From quinn’s paper; Of these antibiotics, four are currently marketed in the U.S. asIMM treatments for clinical mastitis (ampicillin, ceftiofur, cephalothin, penicillin); one isavailable as an intramammary dry cow product (penicillin novobiocin); two are approvedfor IMM treatment but no longer marketed in the U.S. (pirlimycin and erythromycin); one(tetracycline) is labeled for systemic administration in dairy cows but not labeled for thetreatment of mastitis, although extra-label usage is allowed under veterinary supervision;and one (sulfadimethoxine) is only labeled for the treatment of pneumonia and footrotin dairy cows (no extra-label usage of this product is allowed). As few antibiotics areapproved to treat mastitis in U.S. dairy herds, most cases are treated using either first- orthird-generation cephalosporins [1,2]

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