**AMR and virulence in mastitis isolates from conventional and organic dairy farms**

*How to organize?*

* Overall significance
  + Many MIC below clinical breakpoints – so, techinically 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
* Limitations of some of the studies
  + Enumeration/standardization of drug usage
  + Europe vs. US
  + Complicated to compare between
    - Sampling strategies
    - Methodology of determination of antibiograms
      * agar diffusion, broth microdilution
      * the interpretive criteria used for categorizing isolates as susceptible or resistant are based on human data for the majority of compounds tested (Watts and Yancey, 1994; Thornsberry et al., 1997). They cannot be used to predict clinical efficacy and they may not accurately reflect the efficacy of the drug in treatment of bovine mastitis
  + 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
* What else explains degree of AMR carriage? Herd effect – clonality, esp. of contagious organisms
  + Dominant strain aureus may have resistance
  + Different strains associated with carrying resistance?
    - Find literature
  + So, dominant strain in one herd may carry resistance
  + Strain associated with resistance – phylogeny and not just env. pressures
    - From Call 2008: Walk et al. (2007) found that on average organic and conventional dairies have different representation of phylogenetic groupings of E. coli, suggesting there are differences between lineages of E. coli in their ability or probability of assimilating resistance genes
* What else explains degree of AMR carriage? Species effect – carriage of AMR likely associated with species of CNS
  + Older studies not differentiating
  + Literature showing AMR difference by species of NASM?
    - Strep: Rossitto PV, Ruiz L, Kikuchi Y, Glenn K, Luiz K, Watts JL and Cullor JS (2002). Antibiotic susceptibility patterns for environmental streptococci isolated from bovine mastitis in central California dairies. Journal of Dairy Science 85: 132–138.
* Literature showing AB usage = selection pressure
  + - *“Antibiotic resistance is equally likely to diminish in prevalence when antibiotic use is decreased or discontinued. Although individual bacterial strains may retain resistance genes, they are often (gradually) replaced by susceptible strains when the selective pressure is removed”*
      * Phillips I, Casewell M, Cox T, et al. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J Antimicrob Chemother 2004;53:28 –52.
    - *“Generally, percentages of antimicrobial resistance before (conventional) were significantly higher than after (organic) the transition. Overall, percentages of antimicrobial resistant mastitis pathogens decreased after 6 months operating as an organic farm system. An 8-month study was conducted in Thailand to investigate the effects of antimicrobial-resistant patterns of mastitis pathogens during an experimental farm’s 6-month transition from conventional to organic farming. Antimicrobial resistance of mastitis pathogens in the before (conventional) and after (organic) transition periods were compared for 7 antimicrobial drugs used to treat mastitis.”*
      * Suriyasathaporn W. Milk quality and antimicrobial resistance against mastitis pathogens after changing from a conventional to an experimentally organic dairy farm. Asian Austral J Anim Sci May 1, 2010
    - Erskine RJ, Walker RD, Bolin CA, et al. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. J Dairy Sci 2002;85:1111– 8
      * Not much evidence that ***AMR increasing over time,*** which is a different question
      * 7-year study of Michigan dairy herds; the proportion of bacterial isolates susceptible to antibiotics did not change for the majority of tests
      * Overall, the prevalence of AMR over a 7-year period did not change (1994–2000). The prevalence of S. aureus isolates resistant to ampicillin, penicillin and erythromycin declined during this period. Streptococcus uberis isolates became more susceptible to oxacillin, sulfa-trimethoprim gentamicin, and pirlimycin while becoming more resistant to penicillin. Linear declines in AMR were also reported for Streptococcus dysgalactiae, Streptococcus agalactiae, E. coli and Klebsiella pneumoniae. Overall, the authors concluded that there was no indication of increased resistance among mastitis clinical isolates for antimicrobials used commonly to treat mastitis
    - Nam HM, Lim SK, Kang HM, et al. Prevalence and antimicrobial susceptibility of gram-negative bacteria isolated from bovine mastitis between 2003 and 2008 in Korea. J Dairy Sci 2009;92:2020 – 6. 31.
    - Nam HM, Lim SK, Kang HM, et al. Antimicrobial resistance of streptococci isolated from mastitic bovine milk samples in Korea. J Vet Diagn Invest 2009;21:698 –701.
  + BUT ALSO – why 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
* BUT support for using AB
  + Call 2008: decreasing animal health could increase the probability of a higher pathogen load in these animals with commensurate increased risk of exposing humans to genuine pathogens (Cox and Popken, 2006) (also see Claycamp (2006)).
  + It reduces the suffering of animals and prevents pathogenesis in humans via consumption of milkborne/foodborne mastitis pathogens that are potential human pathogens
  + Danger of consumption of raw milk
    - Oliver SP, Boor KJ, Murphy SC, et al. Food safety hazards associated with consumption of raw milk. Foodborne Pathog Dis 2009;7:793– 806.
  + Find papers about pain/inflammation associated with mastitis
    - An Update on the Effect of Clinical Mastitis on the Welfare of Dairy Cows and Potential Therapies
    - Christina S Petersson-Wolfe 1, Kenneth E Leslie 2, Turner H Swartz 3
    - Assessment and Management of Pain in Dairy Cows with Clinical Mastitis Kenneth E. Leslie, DVM, MSca,\*, Christina S. Petersson-Wolfe
    - Ginger L, Ledoux D, Bouchon M, Rautenbach I, Bagnard C, Lurier T, Foucras G, Germon P, Durand D, de Boyer des Roches A. Using behavioral observations in freestalls and at milking to improve pain detection in dairy cows after lipopolysaccharide-induced clinical mastitis.
* Intro material
  + Resistance to antibiotics may be acquired by spontaneously occurring genetic mutations, and be passed vertically by selection to daughter cells. More commonly, resistance is acquired by the horizontal transfer of mobile DNA elements from a donor cell, often from another bacterial species (Chambers, 2001; Sefton, 2002). The two main factors involved in the development of antibiotic resistance in bacteria are the selective pressure by the use of antibiotics and the presence of resistance genes (Levy, 1997; Witte, 2000).
  + There is growing evidence and little doubt that resistance genes can be spread and exchanged between different bacterial populations (McDermott et al., 2002; O’Brien, 2002; Teale, 2002). Resistance that is acquired by horizontal transfer of resistance genes can become rapidly and widely disseminated either by clonal spread of the resistant strain itself or by further genetic exchanges between the resistant strain and other susceptible strains (Chambers, 2001)

***Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland***

*Busato 2000*

* Not a head to head (org. vs. conventional in same study)
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* **Of limited validity? No statistical comparisons actually done; compared organic isolate data to data for conventional isolates from survey 6 yrs previous – no temporal continuity/continuity of methodology/internal validity?**
* Subclinical mastitis; longitudinal study with 2 visits to each farm, once when on pasture and once when in barn; 152 farms and 1907 cows
* Used CMT (quarter-level) to find subclinical mastitis cases to collect and culture
* Mastitis isolates exhibited “comparable” antibiotic resistance frequency as found previously in conventional farms
* Used Kirby-Bauer disk agar-diffusion method for AB susceptibility
* Antibiotic susceptibility was investigated in 123 samples where Streptococci, S. aureus and other coagulase-negative Staphylococci (CNS) could be isolated as single pathogens
* Neomycin resistance of Streptococci was very high and Gentamycin resistance of Streptococci appeared to be higher than in a previously performed survey in conventional farms. On the other hand, antibiotic resistance of S. aureus and of coagulase-negative Streptococci (with the exception of Rifamyin) were similar. (compared 32 strep, 37 SA, 54 CNS to a previous nation-wide survey results describing isolates from conventional swiss farms). Missing test results for a lot of the conventional farm isolates to compare the organic ones to
* Drugs tested
  + Penicillin
  + Ampicillin
  + Cloxacillin
  + Cefalotin
  + Neomycin
  + Gentamicin
  + Tetracyclin
  + Chloramphenicol
  + Erythromycin
  + Cotrimoxacol
  + Ciprofloxacin
  + Rifamycin
  + Clindamycin
* The proportion of bacteria which were resistant to antibiotics was generally higher in this study than in an investigation performed earlier in Switzerland (SchaÈllibaum, 1992, unpublished). There is no indication that a presumably lower use of antibiotics in organic dairy farms has resulted in resistance patterns considerably different from conventional farms. Moreover, it appeared that the proportions of resistant pathogens followed the same tendency of increasing antibacterial resistance as seen in other countries (Krassnig et al., 1997; Myllys et al., 1998). Nevertheless, we caution that only a few samples were analyzed for antibiotic susceptibility. Antibiotics (mostly b-lactam antibiotics and combinations of b-lactams and other antibiotics) were regularly used for dry-cow management in 65% of all study farms, although the regulations for organic dairy production prohibit the application of these substances for other than curative reasons. The relatively frequent use of antibiotic dry-cow treatments indicates, however, that farmers were aware of mastitis problems and that dry-cow treatment with antibiotics is considered an efficient and cost-effective method to control subclinical mastitis

***A Comparison of Antimicrobial Susceptibility Patterns for Staphylococcus aureus in Organic and Conventional Dairy Herds***

*Tikofsky 2003*

* Head to head comparison (org. vs. conventional in same study)
* American organic farms (no AB at all)
* **Cool how they did two different approaches; both strength of association/categorical, and ANOVA/numeric, as many breakpoints were below resistant point; disk diffusion method used mainly in a qualitative manner, placing isolates in either a sensitive, intermediate, or resistant category; this kind of categorization may fail to detect subtle changes in antimicrobial susceptibility within categories. Although clinical relevance is not as clear as classifying isolates as SIR, used zone diameter in millimeters as a quantitative measure to compare relative levels of resistance, to better detect smaller differences between the two groups**
* Hypothesis: If antimicrobial use is the major selective pressure encouraging the development of resistance, then reduced use should result in decreased resistance.
* We compared antimicrobial susceptibility patterns of Staphylococcus aureus isolates obtained from (composite) milk samples from 22 organic (nonantibiotic using) dairy herds to isolates from 16 conventional dairy herds (herds of similar size and geographic distribution; except all used blanket DCT). Susceptibility testing was performed by disk diffusion, and zone diameters were recorded in millimeters for 144 isolates from organic farms and 117 isolates from conventional farms and were also classified as susceptible or not-susceptible (intermediate and resistant categories combined).
* Analysis:
  + Chi-square … high use/low use … proportion susceptible
  + ANOVA: differences in mean zone diameter for isolates from organic vs. conventional
* **Differences in antimicrobial susceptibility were observed between S. aureus isolates from organic and conventional herds for seven of the nine antibiotics studied.** Herds that were certified organic had S. aureus isolates that were more susceptible to antimicrobials. Overall, S. aureus isolates from both organic and conventional herds showed good susceptibility to most commonly used bovine mastitis antimicrobials; however, **isolates from organic herds were significantly more susceptible.**
* “Selective pressure from antibiotic use offers resistant strains a survival advantage.10 Thus, dairy farms constitute an environment where S. aureus is highly prevalent, where antimicrobials are routinely used, and where selective pressure may favor the survival of resistant strains.”
* Kirby-Bauer agar disk diffusion method on Mueller-Hinton agar and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS).
* Antibiotics were chosen based on their activity against Gram-positive cocci and included ampicillin (10 mg), cephalothin (30 mg), erythromycin (15 mg), novobiocin (30 mg), oxacillin (1 mg), penicillin (10 IU), penicillin-novobiocin (10 IU/30 mg), pirlimycin (2 mg), tetracycline (30 mg), and vancomycin (30 mg)
* *Helpful for fourth manuscript?* The strength of association between antibiotic use (conventional or organic) and proportion susceptible or resistant for each antibiotic were evaluated by Chi-square analysis. Where expected counts in the 2 3 2 contingency table were less than 5, a Fisher’s exact test was applied (SAS proc freq v.8.2). Zone diameters for all antibiotics were compared for organic and conventional herds using one-way analysis of variance (ANOVA) (SAS proc Mixed, v. 8.2). Herd was included as a random effect, to account for related observations within a herd. Results for each analysis were deemed significant at p # 0.05. Statistical analyses were performed by the authors at Quality Milk Production Services. Selected correlations between zone diameters were evaluated using Pearson correlation
* Percent susceptible for isolates from both organic and conventional herds for cephalothin, oxacillin, novobiocin, penicillin, novobiocin, and pirlimycin approached 100% and did not differ significantly**. Fewer isolates from the conventional herds were observed in the susceptible range for the remaining antibiotics when compared to isolates from the organic herds: ampicillin (61.5% vs. 80.5%), penicillin (65.8% vs. 79.9%), and tetracycline (87.2% vs. 99.3%).** Percent susceptible for both conventional and organic herds for erythromycin did not differ significantly (49.5% vs. 55.5%) significant differences were observed in the proportion of susceptible isolates from the organic herds compared to conventional herds for ampicillin, penicillin, and tetracycline
* When results were compared on the basis of zone of growth inhibition in millimeters, significant differences were observed for ampicillin, cephalothin, oxacillin, penicillin, penicillin-novobiocin, pirlimycin, and tetracycline. Mean zone diameters were reduced for isolates from conventional herds compared to organic herds
* when mean zone diameter measurements of isolates from the organic and conventional herds were compared, smaller zones of inhibition were observed for conventional farm isolates for six of the nine antibiotics evaluated. In this study, we chose to use zone diameter in millimeters as a quantitative measure of resistance
* Development of antimicrobial resistance is mediated by the presence of genes encoding for resistance. Selective pressure due to antibiotic use influences the expression or acquisition of a resistance phenotype. A close relationship exists between the rate of development of resistance and the quantities of antibiotic used.31
* Resistance may arise either through chromosomal mutation or via the transfer of extrachromosomal elements such as plasmids, integrons, and transposons.26,27,34
* Mastitis is the single most common cause for antibiotic use in dairy cattle
* Amoxicillin and pirlimycin were the most common treatments administered during lactation in our conventional herds; 12 herds treated all clinical cases with antibiotics. DCT was practiced by 100% of the conventional herds; a commercial penicillin-novobiocin intramammary preparation was the most common treatment used
* All of the organic herds in our study had been certified as organic for at least 3 years; most had farmed organically much longer
* The disk diffusion method of determining antimicrobial susceptibility, although widely used and economically attractive, has its limitations. It is used mainly in a qualitative manner, placing isolates in either a sensitive, intermediate, or resistant category, and may fail to detect subtle changes in antimicrobial susceptibility within categories
  + While we recognize concerns associated with the clinical relevance of differences in zone diameter measurements among pathogens categorized as susceptible, it is possible that relative levels of resistance may be compared using zone measurements as a quantitative variable.
* b-Lactams, such as penicillins and cephalosporins, are the most widely used antibiotics in mastitis therapy.37 Their mode of action against Gram-positive bacteria such as S. aureus is via interaction with penicillin-binding proteins (PBPs). PBPs have various functions in the bacterial cell, including cell wall synthesis, b-lactamase activity, and as regulatory proteins.7,9,23 Resistant strains of S. aureus carrying enzymes responsible for the hydrolysis of b-lactams were identified shortly after the introduction of b-lactam antibiotics.6,23,25,29 Various PBPs and b-lactamases have been described.7,9,24 Genes encoding for altered PBPs, penicillinases, and b-lacta- mases may be located chromosomally or on plasmids that may be transferred horizontally, potentially driven by the use of antibiotics. Altered PBPs have a decreased affinity for penicillins, allowing bacterial cell wall synthesis to continue in the presence of b-lactam antibiotics. Intrinsic mechanisms leading to modification of specific penicillin-binding proteins (such as mecA coding for PBP2a) may be involved.9,30 Additionally, more general mechanisms of resistance, such as decreased membrane permeability or membrane-associated active efflux, may also exist.30,41 Combinations of two or more of these mechanisms may explain some of the variability in antibiotic susceptibility within the ‘susceptible’ class seen in this study

***Comparison of Antimicrobial Susceptibility of Staphylococcus aureus Isolated from Bulk Tank Milk in Organic and Conventional Dairy Herds in the Midwestern United States and Denmark***

*Sato 2004*

* Head to head comparison (org. vs. conventional in same study)
* BOTH IN EUROPE, AND US ORGANIC FARMS
* **Overall, antimicrobial susceptibility was very similar between isolates from organic and conventional herds in both countries. Isolates from conventional herds in Wisconsin 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. Differences in antimicrobial susceptibility of isolates between organic and conventional farms were small relative to differences in isolates observed between the US and Denmark.**
* An observational study was conducted to compare the antimicrobial susceptibility patterns of Staphylococcus aureus isolated from bulk tank milk in organic and conventional dairy farms in Wisconsin, United States, and southern Jutland, Denmark. Bulk tank milk samples and data regarding management and production were collected from 30 organic and 30 conventional dairy farms in Wisconsin and 20 organic and 20 conventional dairy farms in Denmark. S. aureus isolates were tested for resistance against 15 antimicrobial agents by semiautomatic microbroth dilution methods in each country. Of the 118 bulk tank milk samples in Wisconsin, 71 samples (60%) yielded at least one S. aureus isolate, and a total of 331 isolates were collected. Of the 40 bulk tank milk samples from Denmark, 27 samples (55%) yielded at least one S. aureus isolate, and a total of 152 isolates were collected. Signi cant differences between organic and conventional dairies were detected only to cipro oxacin in Wisconsin and avilamycin in Denmark. Signi cant differences (P , 0.05) between the two countries were detected in nine antimicrobials. Denmark had a higher probability of having reduced susceptibility to cipro oxacin and streptomycin (P 5 0.015 and 0.003, respectively). Wisconsin isolates had a higher probability of having reduced susceptibility to seven other antimicrobial agents (bacitracin, gentamicin, kanamycin, penicillin, sulphamethoxazole, tetracycline, and trimethoprim). We found small differences between organic and conventional farm types in each country and larger differences between the two national agricultural systems.
* small differences between organic (8.8%) and conventional (14%) farms. Penicillin resistance against CNS isolated from subclinically infected mammary quarters was 48.5% in conventional versus 46.5% in organic herds
* In contrast to organic farms in the United States, antimicrobial drugs can be used for Danish økologisk cows, although the økologisk standards require a threefold longer milk withdrawal time than cows on conventional farms
* US: All 30 organic farms were certi ed by an approved certi cation agency as not using antimicrobials for cows for at least 3 years (mean 5 8.0 years) before the start of this study. For each organic farmer selected, a neighboring ‘‘conventional’’ dairy farm served as a control. All herds were visited twice: once in March and once in September. Management and production data were collected at the  rst visit with the use of questionnaires administered in person
* Denmark: All 30 organic farms were certi ed by an approved certi cation agency as not using antimicrobials for cows for at least 3 years (mean 5 8.0 years) before the start of this study. For each organic farmer selected, a neighboring ‘‘conventional’’ dairy farm served as a control. All herds were visited twice: once in March and once in September. Management and production data were collected at the  rst visit with the use of questionnaires administered in person
* The colonies were examined for purity and identi ed as S. aureus by Gram stain, catalase production, and tube coagulase test (coagulase rabbit plasma with EDTA
* S. aureus isolates were tested for antimicrobial resistance against 15 antimicrobial agents by semiautomatic microbroth dilution methods (Sensititre; Trek diagnostic Systems Inc., Cleveland, Ohio). The MICs of S. aureus were tested for the same antimicrobials and the same range of concentrations in both countries according to manufacturers’ instructions, except that avilamycin was only tested in Denmark and cephapirin was only tested in the Unites States.
* We used the interpretive standards set by the NCCLS (23) and the breakpoints used by DANMAP 2001 (5). Because the MICs for the majority of isolates were far below threshold values for clinical resistance, the MIC for 90% of all isolates tested (MIC90) of each antibiotic was used as a breakpoint on which to dichotomize susceptibility in order to compare the conventional and organic or økologisk farms. Isolates with MICs lower than the MIC90 were categorized as a high-susceptibility isolate; isolates with MICs higher than the MIC90 were categorized as a reduced-susceptibility isolate (Tables 4 and 5). Logistic regression analysis was used to estimate the effect of farm type (organic or økologisk versus conventional) on rates of reduced S. aureus susceptibility to the different antimicrobials. Because the number of tested isolates differ for each farm (0 to 10), the data were considered unbalanced with repeated values for each farm.
* Did not quantify AB usage on farms … in Denmark, Recent work showed that owners of økologisk herds had asked veterinarians to treat their mastitis cows less frequently; however, treatment frequency and antibiotic selection is not substantially different among the two herd types
* The reduced susceptibility to cipro oxacin in conventional dairies relative to organic dairies could be explained by the use of enro oxacin on conventional farms for dairy or beef cattle, by exposure to purchased feeds on conventional farms, or by exposure to other sources of resistant bacteria

***Comparison of Antibiotic Resistance of Udder Pathogens in Dairy Cows Kept on Organic and on Conventional Farms***

*Roesch 2006*

* Head to head comparison (org. vs. conventional in same study)
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* **Percentage of antibiotic resistance did not differ significantly between isolates from cows kept on organic and conventional farms for 12 antimicrobials representing either drugs used to treat mastitis in dairy farms, or drugs important in human medicine. The number of resistant S. aureus strains was numerically higher in isolates from ORG cows vs. CON cows (35% and 18%, respectively), but this different was not statistically significant. NAS had a higher percentage of antibiotic resistance than Staphylococcus aureus.**
* The occurrence of antibiotic resistance was compared between mastitis pathogens (*Staphylococcus aureus*, nonaureus staphylococci, *Streptococcus dysgalactiae, Streptococcus uberis*) from farms with organic and conventional dairy production. **Clear differences in the percentage of antibiotic resistance were mainly species-related**, **but did not differ significantly between isolates from cows kept on organic and conventional farms, except for *Streptococcus uberis*, which exhibited significantly more single resistances (compared with no resistance) when isolated from cows kept on organic farms** (6/10 isolates) than on conventional farms (0/5 isolates).
* Different percentages were found (albeit not statistically significant) in resistance to ceftiofur, erythromycin, clindamycin, enrofloxacin, chloramphenicol, penicillin, oxacillin, gentamicin, tetracycline, and quinupristin-dalfopristin, but, importantly, none of the strains was resistant to amoxicillin-clavulanic acid or vancomycin. Multidrug resistance was rarely encountered.
* Any use of antibiotics leads to a higher risk of resistance selection (Moellering, 1990; Chaslus-Dancla et al., 2000).
* In accordance with the guidelines for Swiss OP farms, the prophylactic use of antibiotic agents in OP farms was lower than in IP farms. This measure should lead to a lower development of antibiotic resistance because a close relationship was found between levels of antibiotic resistance and the exposition to the used antibiotics (Lopez-Lozano et al., 2000)
* Antibiotics of the classes cephalosporins (cefacetril, cefalexin, cefapirin, cefoperazon), aminoglycosides (gentamicin, neomycin, kanamycin), macrolides (spiramycin), lincosamides (lincomycin), β-lactams (amoxicillin, cloxacillin, penicillin), and β-lactam combined with β-lactamase inhibitor (amoxicillin-clavulanic acid) are authorized in Switzerland for the prevention and treatment of mastitis in dairy cows in IP farms.
* Although antibiotic resistance patterns may reflect the antibiotics used for mastitis prevention and treatment in some studies (**Rajala-Schultz et al., 2004**), convincing evidence is lacking that the use of antibiotics for the treatment or prevention of mastitis has resulted in development of resistance to these antibiotics (**Hillerton and Berry, 2005**)
* Typical bacterial species that cause mastitis in dairy cows have few, if any, mechanisms for transfer of resistance to other bacteria, as occurs with intestinal bacteria.
  + *What about NAS and aureus?*
* Organic in Switzerland: The prophylactic use of allopathic drugs or antibiotics is forbidden. It is well known that the therapeutic use of antibiotics should be limited and based on prescription by a veterinarian. For cases of mastitis in OP farms, antibiotics like penicillin, cloxacillin, gentamicin, and neomycin may be used. Because antibiotic use should be low or absent, antibiotic resistance of udder pathogens should not be expected in OP farms or be at least at a lower level than in conventional farms
* 60 certified OP farms with at least 3 yr of organic farming were randomly selected from a pool of interested OP farms. Sixty IP farms, from a pool of interested farms, were selected based on their geographic proximity (ZIP code) to the OP farms; that is, the same agricultural zone (elevation) and farm size (number of cows) as the neighboring farm. On each farm, between 5 and 13 dairy cows (depending on farm size) were randomly selected at 31 d (median) postpartum. In total, 483 OP cows and 487 IP cows were tested.
* Quarters with a CMT ≥ 1+ reaction, but without any clinical signs of mastitis, were considered **subclinical IM**I. Milk samples from quarters diagnosed with a CMT ≥ 2+ reaction were collected (excluded clinical IMI). For cows with 2 or more subclinically infected quarters with the same bacteriological findings (same species), only one isolate was used for the antimicrobial resistance test. Thus, for each bacterial agent, the calculated prevalence based on quarter milk samples reflects the cow-level prevalence
* The minimal inhibitory concentrations of erythromycin, ceftiofur, clindamycin, chloramphenicol, enrofloxacin, gentamicin, tetracycline, vancomycin, oxacillin, penicillin, and the combinations quinupristin-dalfopristin and amoxicillin-clavulanic acid were determined in Mueller-Hinton broth for staphylococci, and in Mueller-Hinton broth supplemented with 5% horse blood for streptococci using custom sensititre susceptibility plates, and according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2002). The breakpoints determining resistance were those recommended in the CLSI guidelines M31-A2 (CLSI, 2002) and M7-A6 (CLSI, 2003). Strains showing intermediate category were classified as resistant (Tables 1 to 4). Only staphylococci, not streptococci, were tested for resistance to oxacillin
  + The 12 antibiotics tested are representatives of the classes of antibiotics used to treat mastitis in dairy farms (aminoglycosides, cephalosporins, macrolides, lincosamides, β-lactams combined with or without β-lactamase inhibitor), or are important antibiotics used in human medicine (cephalosporins, fluoroquinolones, clindamycin, erythromycin, quinupristin-dalfopristin, gentamicin, penicillin, tetracyclines, vancomycin)
* 158 isolates of quarter milk samples (93 from OP and 65 from IP cows) from quarters diagnosed with a CMT ≥ 2+ reaction were tested for antibiotic resistance. Of these, 79 isolates (46 from OP and 33 from IP cows) were identified as Staphylococcus aureus, 38 (19 OP, 19 IP) as nonaureus staphylococci, 28 (19 OP, 9 IP) as Streptococcus uberis, and 13 (9 OP, 4 IP) as Streptococcus dysgalactiae
  + Aureus
    - The percentage of resistance to enrofloxacin in isolates from IP and OP farms was not different. Resistance percentages to chloramphenicol, gentamicin, and penicillin were not different in isolates from OP and IP cows. Resistance to ceftiofur, clindamycin, erythromycin, oxacillin, quinupristin-dalfopristin, and tetracycline was found only in isolates from OP cows
    - resistant Staphylococcus aureus strains toward antibiotic agents was slightly higher in isolates from OP than IP cows (35 and 18%, respectively).
    - The higher number of multidrug-resistant Staphylococcus aureus strains in OP than in IP farms (although not significant) was unexpected
  + NAS
    - All nonaureus Staphylococcus isolates (n = 38) were susceptible to amoxicillin-clavulanic acid, ceftiofur, enrofloxacin, and vancomycin (Table 2). Resistance percentages to oxacillin and penicillin were not different in isolates from IP and from OP cows. Resistance to chloramphenicol, gentamicin, quinupristin-dalfopristin, and tetracycline was only found in isolates from OP cows. On the other hand, resistance to erythromycin was only observed in strains from IP cows. The resistance percentage to clindamycin in isolates from both OP and IP cows was not different
    - Nonaureus staphylococci had a higher percentage of antibiotic resistance than Staphylococcus aureus, which was in agreement with previously reported data of Corti et al. (2003). The number of nonaureus staphylococci that showed resistance to at least one antibiotic was higher than that described by Rajala-Schultz et al. (2004)
    - The values for penicillin resistance of OP as well as in IP strains were higher than those reported by Busato et al. (2000; 13%), but comparable to Corti et al. (2003; 31%) and Rajala-Schultz et al. (2004; 32%). However, resistances were lower than described in other European and US studies (Owens et al., 1997; Myllys et al., 1998).
  + Uberis
    - All isolates (n = 28) were susceptible to amoxicillinclavulanic acid, ceftiofur, tetracycline, and vancomycin (Table 3). Resistance percentages to chloramphenicol, enrofloxacin, gentamicin, and penicillin were not different in isolates from OP than from IP cows. Resistance to clindamycin, erythromycin, and quinupristin-dalfopristin was only found in strains from OP cows.
    - The number of tested Streptococcus uberis isolates was very small. Higher antibiotic resistance percentages for most antibiotics, except penicillin, were found in isolates from OP cows than from IP cows
  + Dysgalactiae
    - All isolates (n = 13) were susceptible to amoxicillinclavulanic acid, ceftiofur, chloramphenicol, quinupristin-dalfopristin, and vancomycin (Table 4). Resistance percentages to enrofloxacin, gentamicin, and tetracycline were not different in isolates from IP or OP farms. Resistance to clindamycin, erythromycin, and penicillin was only observed in isolates from IP cows.
    - In OP farms, the total number of antibiotic resistant Streptococcus dysgalactiae isolates (9) was lower than that of Streptococcus uberis (27). No tetracycline-resistant Streptococcus uberis strain was found in both IP and OP farms, whereas 50% of the Streptococcus dysgalactiae isolates displayed tetracycline resistance
  + Multidrug resistances against up to 8 different antibiotic agents were observed; however, the isolates of the 4 bacterial groups tested were most frequently resistant against just 1 or 2 antibiotics (Table 5). The resistance percentages were not significantly different among bacterial strains isolated from OP and IP cows with the exception of Streptococcus uberis isolates, which exhibited more single resistances (compared with no resistance) when isolated from cows kept on OP farms than on IP farms (6 of 10 isolates vs. 0 of 5 isolates, respectively; P = 0.044).
  + The percentage of antibiotic resistance was not different among Staphylococcus aureus, Streptococcus uberis, or Streptococcus dysgalactiae isolates from OP than IP cows, except for β-lactam antibiotics. Overall, the frequency of antibiotic resistance did not differ between isolates from OP and IP cows, but differences were found among bacterial species

***Resistance to penicillin of Staphylococcus aureus isolates from cows with high somatic cell counts in organic and conventional dairy herds in Denmark***

*Bennedsgaard 2006*

* Head to head comparison (org. vs. conventional in same study)
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* **Interesting bc they have herd grps both CON and ORG, but also herds in different stages of transition; talk about herd effect/clonality of SA carrying resistance based on genotype**
* Quarter milk samples from cows with high risk of intramammary infection were examined to determine the prevalence of Staphylococcus aureus (SA) and penicillin resistant SA(SAr) in conventional and organic dairy herds and herds converting to organic farming in a combined longitudinal and cross-sectional study
* 20 conventional herds, 18 organic herds that converted before 1995, and 19 herds converting to organic farming in 1999 or 2000 were included in the study. Herds converting to organic farming were sampled three times one year apart; the other herds were sampled once. Risk of infection was estimated based on somatic cell count, milk production, breed, age and lactation stage. The prevalence of penicillin resistance among SA infected cows was 12% (95% confidence interval: 6%–19%) when calculated from the first herd visits. No statistically significant differences were observed in the prevalence of SAr or the proportion of isolates resistant to penicillin between herd groups. The proportion of isolates resistant to penicillin was low compared to studies in other countries except Norway and Sweden. Based on the low prevalence of penicillin resistance of SA, penicillin should still be the first choice of antimicrobial agent for treatment of bovine intramammary infection in Denmark
* Often a few clones of SA dominate in the single herd due to the contagious nature of the bacteria. Therefore, surveys only including few herds might provide invalid estimates of the general prevalence
* The conventional and old organic herds were sampled once. In all herds, quartermilk samples were collected from 30 cows with high somatic cell counts. The criteria for sampling were an esti-mated risk of infection based on the history of SCC, breed, and calving number of the individual cow
* Isolation of penicillin resistant SA in at least one quarter milk sample from a cow was used as outcome as a binary variable with the logit link function. Herd was introduced as a random variable in a hierarchical model. A categorical variable for the five herd groups: conventional, old organic, converting herds year 0, converting herds year 1 and converting herds year 2 was introduced to test differences between herd groups.
* Milk production and the prevalence of mastitis treatment in the conventional group were significantly higher than in the old organic and the converting herds after one year of organic production; No significant differences were found in the prevalence of SAr between the herd groups.
* The large proportion of herds with no penicillin resistant SA isolates indicates that the occurrence of SAr must be seen as a herd problem at the present low overall prevalence of resistant isolates
  + More a function of herd-level what strain is dominant than org vs. conventional mgmt.
  + “The resistant isolates found in single herds probably represent the same clone”
  + Strain typing could help inform this
* No difference in prevalence of penicillin resistant SA or in the proportion of SA resistant to penicillin was found between conventional and old organic herds or before and after converting to organic farming.

***Relationship Between Antimicrobial Drug Usage and Antimicrobial Susceptibility of Gram-Positive Mastitis Pathogens***

*Pol and Ruegg 2007*

* Head to head comparison (org. vs. conventional in same study)
* American organic farms (no AB at all)
* Exposure to selected antimicrobial drugs (n = 10) was standardized by calculation of the number of defined daily doses used per cow. Farms (n = 40) were categorized based on amount of antimicrobial exposure: organic (no usage); conventional–low usage (conventional farms not using or using less than or equal to the first quartile of use of each compound); and conventional–high usage (conventional farms using more than the first quartile of a particular compound). The minimum inhibitory concentration (MIC) of selected antimicrobial drugs was determined using a commercial microbroth dilution system for isolates of Staphylococcus aureus (n = 137), coagulase-negative staphylococci (CNS, n = 294), and Streptococcus spp. (n = 95) obtained from subclinical mastitis infections
* Most isolates were inhibited at the lowest dilution tested of most antimicrobial drugs. Survival curves for Staph. aureus and CNS demonstrated heterogeneity in MIC based on the amount of exposure to penicillin and pirlimycin. For CNS, farm type was associated with the MIC of ampicillin and tetracycline. For Streptococcus spp., farm type was associated with MIC of pirlimycin and tetracycline. For all mastitis pathogens studied, the MIC of pirlimycin increased with increasing exposure to defined daily doses of pirlimycin. The level of exposure to most other antimicrobial drugs was not associated with MIC of mastitis pathogens. A dose–response effect between antimicrobial exposure and susceptibility was observed for some pathogen–antimicrobial combinations, but exposure to other antimicrobial drugs commonly used for prevention and treatment of mastitis was not associated with resistance
* In the United States, a limited number of antimicrobial drug groups are available for intramammary treatment of mastitis including β-lactams (penicillin, cephapirin, ceftiofur, amoxicillin, hetacillin, and cloxacillin), macrolides (erythromycin), coumarines (novobiocin), and lincosamides (pirlimycin) (FDA–Center for Veterinary Medicine, 2004)
* The previously described studies (Tikofsky et al., 2003; Rajala-Schultz et al., 2004) have contributed to our understanding of possible relationships between antimicrobial usage and bacterial resistance in dairy cattle, but none have quantified antimicrobial usage at the farm or cow level. The objective of this study was to analyze the relationships between a standardized measure of antimicrobial exposure on dairy farms and results of susceptibility testing of a variety of mastitis pathogens.
* Commercial organic (ORG; n = 20) and conventional (CON; n = 20) farms were selected. Enrollment criteria required herds to have a 6-mo average bulk tank SCC ≥250,000 cells/ mL. Additionally, CON farms were required to have used comprehensive antimicrobial dry-cow therapy (DCT) for at least 5 yr. Organic farms were required to be certified organic for at least 3 yr. Antimicrobial exposure was estimated using an 84- question survey instrument. Antimicrobial usage was standardized using a defined daily dose (DDD). In brief, the DDD is the maximum dose a standard animal (BW = 680 kg) would receive if it were treated following the FDA-approved label dosages. For each farm, the number of DDD used at farm level was calculated by dividing the reported total dose (mg or IU) of each drug used per year by the DDD of that antimicrobial drug. The number of DDD was divided by the total number of milking cows to estimate the density of use of the antimicrobial drug. Antimicrobial drug usage density was expressed as number of DDD per lactating cow per year. The density of use was expressed as number of DDD used per cow (lactating and nonlactating) per year. The total exposure to antimicrobial drugs was estimated per farm per year for treatment of selected diseases. Farms were categorized into 1 of 3 usage groups based on density of antimicrobial drug usage for each drug studied: 1) Organic farms: no use of antimicrobial drugs (n = 20); 2) Conventional farms, low exposure (CONL; n = 15): no use of the studied antimicrobial drug or used less DDD than the 5 farms with the highest number of DDD; and 3) Conventional farms, high exposure (CON-H; n = 5): the 5 farms that reported the highest DDD for each compound among the studied farms
* Quarter milk samples were obtained from a maximum of 50 multiparous cows that had no signs of clinical mastitis during a single visit to each farm. Only multiparous cows were sampled to ensure that at least 1 known exposure to intramammary antimicrobial drugs (DCT) had occurred for all sampled animals
* Antimicrobial susceptibility was evaluated for isolates confirmed as: Staph. aureus, CNS, and Strep. spp. (except Strep. agalactiae). For both CON and ORG herds, a similar variety of CNS species were included in susceptibility testing. Definite identification of grampositive bacteria suspected of belonging to the genera Staphylococcus or Streptococcus was performed using a miniaturized identification method (BBL Crystal, Becton Dickinson Microbiology Systems, Franklin Lakes, NJ). The majority of streptococci included in susceptibility testing were Streptococcus uberis and Streptococcus dysgalactiae; no isolates identified as enterococci were included in the susceptibility testing. Antimicrobial susceptibility was tested using the mastitis panel of a commercial broth microdilution test (Sensititre, TREK Diagnostics, Cleveland, OH) following guidelines of the Clinical and Laboratory Standards Institute
* Antimicrobial drugs tested were ampicillin, ceftiofur, cephalothin, erythromycin, oxacillin + 2% NaCl, penicillin, penicillin/novobiocin, pirlimycin, sulfadimethoxine, and tetracycline.
* For each farm and isolate type, isolates included in statistical analysis were randomly selected. Moreover, only 1 isolate per cow and no more than 20 isolates per farm were included in the analysis to avoid statistical dependence
* **Is MIC independent of herd type (CON vs. ORG)?**
* **Is proportion of susceptible isolates independent of herd type (CON vs. ORG)?**
  + Susceptibility outcomes were placed into 2 categories that formed the columns: susceptible and resistant. The resistant category included those isolates categorized as either intermediate or resistant.
* More samples (58.4%) obtained from CON farms yielded no growth compared with samples obtained from ORG farms (44.4%). The prevalence of all mastitis pathogens, except coliforms, was greater for ORG farms compared with CON farms
* Of the total mastitis pathogens isolated, significant differences in the proportion of pathogens based on farm type were observed for CNS (38% CON and 30% ORG)
  + No significant differences were found in the proportion of Staph. aureus isolated based on herd type (8.5% of isolates)
* The number of Staph. aureus isolates per farm used in statistical analysis ranged from 1 to 9 in CON herds, whereas in ORG herds it ranged from 1 to 18 due to the limitation of only 1 isolate per cow and ≥20 per farm. A total of 52 and 85 Staph. aureus obtained from CON (n = 15) and ORG (n = 18) farms, respectively, were used for statistical analysis. The number of CNS isolates per farm used in statistical analysis ranged from 2 to 16 in CON herds, whereas in ORG herds it ranged from 1 to 16. A total of 160 and 135 CNS obtained from CON (n = 20) and ORG (n = 19) farms, respectively, were used for statistical analysis. The number of Strep. spp. isolates per farm used in statistical analysis ranged from 1 to 5 in CON herds, whereas in ORG herds it ranged from 1 to 7. A total of 42 and 53 Strep. spp. obtained from CON (n = 17) and ORG (n = 19) farms, respectively, were used for statistical analysis.
* Farm type was associated with the MIC of pirlimycin for Staph. aureus (χ2 = 9.4; P = 0.009), and with the MIC of sulfadimethoxine for Staph. aureus (χ2 = 8.6; P = 0.033). Farm type was not associated with the MIC of the other antimicrobial drugs tested with Staph. aureus (P > 0.18). Staph. aureus isolates obtained from CON herds were more likely to be resistant to ampicillin (odds ratio = 7.7; P = 0.003) and penicillin (odds ratio = 6.3; P = 0.01) compared with isolates obtained from ORG herds. Farm type was associated with the MIC of ampicillin (χ2 = 12.1; P = 0.029), MIC of pirlimycin (χ2 = 42.7; P < 0.001), and MIC of tetracycline (χ2 = 12.9; P = 0.001). Farm type was not associated with the MIC of the other antimicrobial drugs tested using CNS (P > 0.09). Coagulase-negative staphylococci isolates obtained from CON herds were more likely to be resistant to ampicillin (odds ratio = 2.4; P = 0.009), penicillin (odds ratio = 2.2; P = 0.01), pirlimycin (odds ratio = 29.4; P < 0.0001), and tetracycline (odds ratio = 9.4; P = 0.0004) than those from ORG. Farm type was associated with the MIC of pirlimycin (χ2 = 16.17; P < 0.001), and with the MIC of tetracycline (χ2 = 157; P = 0.001). Farm type was not associated with the MIC of the other antimicrobial drugs tested with Strep. spp. (P > 0.19). Streptococcus spp. isolates obtained from CON herds were more likely to be resistant to pirlimycin (odds ratio = 14.1; P = 0.002) and tetracycline (odds ratio = 6.1; P = 0.0003)
* The farms enrolled in this study presented different levels of exposure to antimicrobial drugs as demonstrated by differences in DDD among farms and the variety of antimicrobial drugs used in CON dairy farms to treat clinically or subclinically diseased animals and the minimal exposure to antimicrobial drugs in ORG farms. The mean time since ORG farms were certified organic was 7.6 yr. 1 ORG producer reported the use of DCT in a few quarters. No other uses of antimicrobial drugs were reported by ORG farmers. Therefore, antimicrobial exposure in ORG dairy farms may be considered minimal.
* Our study estimated exposure to DCT during the previous 5 yr and exposure to other antimicrobial drugs used for treatments during the previous 2 yr
* Antimicrobial susceptibility data may be categorized as susceptible–intermediate–resistant (SIR) or may be expressed as the MIC. The MIC is the lowest concentration of antimicrobial agent that completely inhibits bacterial growth (Prescott et al., 2000; Clinical and Laboratory Standards Institute, 2002). The susceptible–intermediate–resistant categories are based on MIC. breakpoints defined by CLSI. The MIC provides more continuous data (ordinal data) that might reflect better the differences among groups. MIC, rather than proportion of susceptible, was more useful for monitoring subtle changes relative to antimicrobial drug exposure
* *“Comparison of antimicrobial resistance of mastitis pathogens among studies is difficult because of differences in methodology”*
* The proportion of resistant isolates was generally small and for most antimicrobial drugs, fewer resistant isolates were obtained from ORG farms. Nevertheless, a high proportion of isolates resistant to sulfadimethoxine were observed in both farm types. The proportion of Strep. spp. isolates that were resistant to sulfadimethoxine was greater for isolates obtained from ORG herds as compared with isolates obtained from CON herds
* A dose–response relationship between the antimicrobial exposure and resistance is another important causal criterion. Our study demonstrated a dose–response effect for several antimicrobial drug exposures and the MIC of the studied pathogens
* Our study did not detect a reduced risk of antimicrobial resistance in the farms that have been organic for a longer period compared with the farms that have been organic for a shorter period
* Use of 2 compounds commonly administered for treatment of IMI (penicillin and pirlimycin) was associated with resistance of mastitis pathogens, but use of many other commonly used compounds was not. A dose–response effect between pirlimycin usage groups and pirlimycin MIC was observed for all isolates studied. The use of penicillin was associated with reduced susceptibility of Staph. aureus and CNS isolates. However, the use of cephapirin (a widely used antimicrobial drug for IMI treatments) was not associated with reduced susceptibility of any of the studied pathogens

***Comparison of types and antimicrobial susceptibility of Staphylococcus from conventional and organic dairies in west-central Minnesota, USA***

*Bombyk 2007*

* Head to head comparison (org. vs. conventional in same study)
* American organic farms (no AB at all)
* Organic livestock operations therefore offer a useful setting for comparisons of bacterial antimicrobial resistance in environments with differing antimicrobial usage
* CNS could potentially provide a reservoir of resistance genes for the more contagious pathogens
* To assess whether conventional and organic dairy management practices are associated with differences in the susceptibility of Staphylococcus to antimicrobial agents. Staphylococcus was isolated from milk samples collected from conventional and organic dairies in west-central Minnesota. Isolates were categorized as (1) coagulase-positive, (2) novobiocin-sensitive coagulase-negative or (3) novobiocin-resistant coagulase-negative … a characteristic that can be used to subcategorize CNS of bovine origin. Novobiocin-resistant coagulase-negative Staphylococcus (CNS) was more common on conventional farms and novobiocin-sensitive CNS predominated the isolates from organic farms. Overall, a larger proportion of isolates from organic rather than conventional farms were susceptible to erythromycin, pirlimycin and tetracycline. However, for pirlimycin and tetracycline, different patterns of susceptibility were observed among Staphylococcus categories. In this study, organic dairy management was associated with more overall antimicrobial susceptibility among Staphylococcus than was conventional management. However, different patterns of susceptibility among Staphylococcus categories suggest that multiple management practices, including some unrelated to antimicrobial use, may contribute to the observed differences in susceptibility
* Aminocoumarins are very potent inhibitors of bacterial DNA gyrase and work by targeting the GyrB subunit of the enzyme involved in energy transduction
* Eight of the dairies had been certified for at least 1 year under the standards of the USDA National Organic Program (http://www.ams.usda.gov/nop/NOP/standards. html, accessed 15 January 2007) and are termed ‘organic’ in this study. The other eight dairies are termed ‘conventional’ because they were neither certified nor seeking to follow organic management practices. Composite milk samples were collected from all healthy cows
* confirmed as Staphylococcus by Gram stain (Gram-positive cocci), catalase testing (positive) and thioglycollate growth pattern (facultative and nonmotile). Confirmed Staphylococcus isolates were identified as coagulase-positive or coagulase-negative by a tube coagulase test. The novobiocin susceptibility of coagulasenegative isolates was determined by agar disc diffusion and interpreted by breakpoints according to guidelines established by the Clinical and Laboratory Standards Institute (formerly NCCLS) for bacteria isolated from animals
* Susceptibility to cefoxitin [30 lg; for determination of methicillin resistance (Velasco et al. 2005)], cephalothin (30 lg), erythromycin (15 lg), novobiocin (5 lg; for identification purposes only), penicillin (10 IU), pirlimycin (2 lg), tetracycline (30 lg) and vancomycin (30 lg) was determined.
* Staphylococcus isolates were identified as susceptible or not susceptible (intermediate or resistant) to each antimicrobial agent. Chi-squared analysis was used to compare populations, and the Rao– Scott second-order correction was used to account for clustering by farm (Rao and Scott 1992). The statistical analysis was performed using the ‘svydesign’ and ‘svychisq’ functions within the r software package
* Milk samples were collected from 339 conventionally managed cows and 501 organically managed cows on sixteen total dairies (eight of each management type). Owners of organically managed cows confirmed that they had not used antimicrobial agents for at least 4 years (at least 1 year of certification and 3 years of transition). Owners of conventionally managed cows all reported usage of several antimicrobial drugs in the past year: cephalosporins (seven owners), penicillins (six owners), tetracyclines (five owners) and pirlimycin (five owners). Five owners of conventionally managed cows reported practicing dry cow therapy, the administration of antimicrobial drugs to all cows at the end of lactation
* NRCNS (resistant) predominated the isolates from conventionally managed cows while NSCNS (susceptible) predominated those from organic farms
* A larger proportion of isolates from organic farms were susceptible to pirlimycin and tetracycline compared with those from conventional farms. Susceptibility to erythromycin and penicillin did not differ significantly by farm type
* susceptibility is broken down by category of Staphylococcus (novobiocin susceptible or resistant): Isolates within both CNS categories from organic farms were more likely to be susceptible to pirlimycin than CNS from conventional dairies. However, NSCNS isolates were more likely to be susceptible to tetracycline than NRCNS isolates for both farm types; no difference in tetracycline susceptibility was seen between farm types within either CNS category. No significant patterns emerge for erythromycin or penicillin susceptibility when broken down in this way
* Our data show that **organic and conventional management practices are associated with significantly different profiles of Staphylococcus in dairy cows** (CNS that were either susceptible or resistant to novobiocin) --- different mgmt. 🡺 difference in dominant species of CNS (a proxy - probably)
  + A previous study of conventionally raised dairy cows found more NRCNS in the udders of animals kept in confinement and more NSCNS in those kept on pasture (White et al. 1989). This may be because, for much of the day, cows in confinement may be exposed to bedding and hay often containing environmental Staphylococcus, which are predominantly NRCNS (Matos et al. 1991). Organic management requires that livestock be kept on pasture for parts of each day (weather permitting), so this management difference may contribute to the different profiles of CNS in our study
* All of the Staphylococcus isolates in this study were susceptible to vancomycin and methicillin (as tested by cefoxitin susceptibility), antimicrobial agents important to human medicine, but not used on any of the dairies in this study. Interestingly, **all isolates were also susceptible to cephalothin**, **although all but one of the owners of conventionally managed cows reported using cephalosporin antimicrobials in the past year**. This result is similar to those of other recent studies that have found high levels of susceptibility to cephalosporins among Staphylococcus (Tikofsky et al. 2003; Rajala-Schultz et al. 2004), despite the fact that cephalosporin antimicrobials are commonly used on US dairies (Zwald et al. 2004).
* in the case of susceptibility to pirlimycin (Table 3, Panel A), an antimicrobial agent used only on dairy farms, this could be the case. On organic farms, where pirlimycin has not been used for several years, a larger proportion of isolates from both CNS categories were susceptible to pirlimycin when compared with isolates from conventional farms, where five of eight owners reported using pirlimycin. This is in agreement with the recent study by Pol and Ruegg (2007), in which they reported a correlation between pirlimycin use and increased MIC values for pirlimycin among several mastitis pathogens, including CNS
* tetracycline resistance – one type of CNS more susceptible to tetracyclines – this type was dominant on organic farms – so, a function of dominance of this type of CNS, not really organic status

***Reproductive Performance, Udder Health, and Antibiotic Resistance in Mastitis Bacteria isolated from Norwegian Red cows in Conventional and Organic Farming***

*Garmo 2010*

* Head to head comparison (org. vs. conventional in same study)
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* The objectives of this study were to investigate whether there were differences between Norwegian Red cows in conventional and organic farming with respect to antibiotic resistance in udder pathogens
* 25 conventional and 24 organic herds from south-east and middle Norway participated in the study. Herds were matched such that geographical location, herd size, and barn types were similar across the cohorts. Herds were matched with the organic farms according to herd size (± five cow-years) and type of housing. All organic herds were certified as organic between 1997 and 2003 (The organic farms converted at least four years before the start of the study). The herds were visited once during the study
* There was few S. aureus isolates resistance to penicillin in both management systems. Penicillin resistance against Coagulase negative staphylococci isolated from subclinically infected quarters was 48.5% in conventional herds and 46.5% in organic herds
* In Norway, the use of synthetic veterinary products prophylactically is prohibited. Withdrawal times for prescribed products are twice as long as corresponding time periods for conventional farming. During one year a maximum of three treatments periods with drugs are allowed for each individual
* Each herd was visited once by the same researcher between February and June in 2006. During this visit quarter milk samples for bacteriological examination were collected aseptically from 523 conventional and 487 organic lactating cows. The farmers were requested to collect quarter milk samples from all cows affected by clinical mastitis
* Suspected staphylococcal colonies were tested using the tube coagulase test; Staphylococci were differentiated from streptococci with a catalase test. Staphylococcal isolates were tested for b-lactamase activity by the cloverleaf method using Staphylococcus aureus ATCC 25923 as the indicator strain
* Of the staphylococci isolated from milk samples of subclinically infected quarters collected during the routine visits to the conventional herds, 81 of the 167 isolates (48.5%) of coagulase-negative staphylococci (CNS) and 6 of the 68 isolates (8.8%) of S. aureus were resistant to penicillin. The corresponding findings in the organic herds were for CNS 93 penicillin-resistant isolates out of 200 (46.5%) and for S. Aureus nine penicillin-resistant isolates out of 64 (14.0%). Of the isolates from quarters with clinical mastitis, resistance to penicillin was not found among the 59 S.aureus from conventional and organic herds. Whereas one out of one of the CNS isolates from the organic herds, and three out of five of the CNS isolates from conventional herds were penicillin-resistant… **no statistical analyses on this**

***Short communication: Prevalence of methicillin resistance in coagulase-negative staphylococci and Staphylococcus aureus isolated from bulk milk on organic and conventional dairy farms in the United States***

*Cicconi-Hogan 2014*

* Head to head comparison (org. vs. conventional in same study)
* American organic farms (no AB at all)
* The objective of this study was to evaluate the presence of methicillin-resistant Staphylococcus aureus and coagulase-negative Staphylococcus spp. in bulk tank milk samples from 288 organic and conventional dairy farms located in New York, Wisconsin, and Oregon from March 2009 to May 2011. Due to recent publications reporting the presence mecC (a mecA homolog not detected by traditional mecA-based PCR methods), a combination of genotypic and phenotypic approaches was used to enhance the recovery of methicillin-resistant organisms from bulk tank milk. In total, 13 isolates were identified as methicillin resistant (positive for mecA): Staph. aureus (n = 1), Staphylococcus sciuri (n = 5), Staphylococcus chromogenes (n = 2), Staphylococcus saprophyticus (n = 3), Staphylococcus agnetis (n = 1), and Macrococcus caseolyticus (n = 1). 7 were from conventional farms, 6 from organic. The single methicillin-resistant Staph. aureus isolate was identified from an organic farm in New York, for an observed 0.3% prevalence at the farm level. The methicillin-resistant coagulase-negative staphylococci prevalence was 2% in the organic population and 5% in the conventional population. We did not identify mecC in any of the isolates from our population. Of interest was the relatively high number of methicillin-resistant Staph. sciuri recovered, as the number of isolates from our study was considerably higher than those recovered from other recent studies that also assessed milk samples
* Staphylococcus sciuri was predominant among our MRCNS isolates (6 isolates out of 12 MRCNS
* Our research suggests that the presence of a potential methicillin-resistant Staphylococcus reservoir in milk, and likely the dairy farm population in the United States, is independent of the organic or conventional production system.
* Methicillin-resistant Staph. aureus (MRSA) is of major concern in the human population, as it is difficult to treat. The mecA gene confers methicillin resistance by encoding a penicillin-binding protein, known as PBP-2α. It is located on a mobile element called staphylococcal cassette chromosome, known as SCCmec, which allows other species of Staphylococcus to pick up the methicillin resistance
* The conventional farms were included in the study based on proximity to the organic farms and were matched based on herd size category (0–99 adult cows, 100–199 adult cows, or ≥200 adult cows). Six bulk tank milk samples were collected from each farm at the time of the visit. A genotypic approach (Virgin et al., 2009) was initially used to determine if the nuc gene, which encodes the thermostable nuclease of Staph. aureus (Brakstad et al., 1992), and a 174-bp portion of the mecA gene (Martineau et al., 2000), were present in the isolates cultured from the bulk tank milk … a phenotypic approach was used to enhance the recovery of MRSA and MRCNS from the milk and to identify methicillin-resistant genotypes other than those that could be identified with the previously described PCR protocol … enrichment and MRSA selective plates. All isolates from both approaches were identified using 16S rRNA and rpoB speciation.
* Of the 11 MRCNS isolates that were detected, 3 were isolated from a single farm, 3 were isolated from another farm, and the remaining isolates were from unique farms. In total, isolates were detected from 9 unique farms for a farm-level prevalence of 3% (exact CI: 1.4–5.6%). The 2 Staphylococcus chromogenes isolates found on farm 3 were RAPD typed and were determined to be different isolates.

***Short communication: Methicillin-resistant Staphylococcus aureus in conventional and organic dairy herds in Germany***

*Tenhagen 2018*

* Head to head comparison (org. vs. conventional in same study)
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* Methicillin-resistant Staphylococcus aureus (MRSA) have been described repeatedly in dairy herds. In this study, we compared the prevalence and antimicrobial resistance of MRSA in bulk tank milk from conventional and organic dairy herds in Germany. Samples were collected from 372 conventional and 303 organic dairy herds throughout Germany. Bulk tank milk (25 mL) was tested for MRSA using an established double selective enrichment method. The MRSA isolates were typed using spa typing and tested for resistance to 19 antimicrobials using the broth microdilution method. The prevalence of MRSA was higher in BTM samples from conventional dairy herds (9.7%) compared with samples from organic herds (1.7%, P< 0.01) Herd size and region were associated with differences in prevalence. Isolates from conventional herds tended to be more resistant to antimicrobials; however, because of the limited number of isolates from organic herds, no statistical tests were performed.
* Selection of herds was based on a national sampling plan designed to cover the different German federal states (Länder) according to their share of the national conventional and organic dairy cow population. On the national level, the number of herds to be sampled was calculated for each region based on the distribution of cows rather than herds to account for substantially different distributions of herd sizes in different regions in Germany (i.e., smaller herds in the south and large herds in the east). However, farm size was not considered when choosing herds within a state. Data on the distribution of dairy cows were provided by the national bureau of statistics (www.destatis.de). Minimum herd size was 20 lactating cows
* examined using a harmonized double selective enrichment protocol, as described previously (Tenhagen et al., 2014), within 48 h of arrival at the laboratory. Presumptive MRSA (one randomly chosen isolate from the selective agar or sample) were submitted to the National Reference Laboratory (NRL; Berlin, Germany) for coagulase-positive staphylococci, including Staph. aureus, where isolates were confirmed as MRSA by an in-house multiplex PCR simultaneously targeting the 23S rDNA gene specific for Staphylococcus species (Straub et al., 1999), the nuclease gene nuc, which is specific for Staph. aureus, and the resistance gene mecA (Poulsen et al., 2003). Isolates of Staph. aureus that were resistant to cefoxitin but negative for mecA would have additionally been tested for mecC but no isolate fulfilled these criteria.
* The antimicrobial susceptibility of Staph. aureus was examined by broth microdilution according to the guidelines of Clinical and Laboratory Standards Institute (Wayne, PA) at the NRL for antimicrobial resistance and included 19 different antimicrobial substances
* comparative data on the extent of antimicrobial use in organic and conventional dairy herds are not available for Germany
* seems to be effect of herd size and region; model controls for region and herd size – conv vs. organic still significant, but herd size and region are as well

***Antimicrobial susceptibilities in dairy herds that differ in dry cow therapy usage***

*McDougall 2020*

* Head to head comparison (org. vs. conventional in same study)
* American organic farms (no AB at all)
* This cross-sectional, observational study determined the minimum inhibitory concentrations (MIC) of 10 antimicrobials for co-agulase-negative staphylococci (CNS), Staphylococcus aureus, Streptococcus dysgalactiae, and Streptococcus uberis isolates from milk samples from dairy cows with somatic cell counts >200,000 cells/mL in herds that had been organic for >3 yr (n = 7), or had used either ampicillin-cloxacillin DCT (n = 11) or cephalonium DCT (n = 8) in the preceding 3 yr
* The MIC distributions of isolates from different herd types were compared using binomial or multinomial logistic regression. Of 240 CNS isolates, 12.9, 0.8, 7.1, 32.6, and 1.2%, were intermediate or resistant to ampicillin, cephalothin, erythromycin, penicillin, and tetracycline, respectively. Of 320 Staph. aureus isolates, 29.0, 2.5, 1.2, and 34.9% were intermediately resistant or resistant to ampicillin, penicillin, erythromycin, and oxacillin, respectively. Of 184 Strep. uberis isolates, 1.1, 25.0, 1.6, and 1.6% were intermediately resistant or resistant to erythromycin, penicillin, pirlimycin, and tetracycline, respectively. Generally, the MIC of CNS and streptococcal isolates from organic herds were lower than isolates from herds using DCT. However, the differences in MIC distributions occurred at MIC below clinical breakpoints, so that the bacteriological cure rates may not differ between isolates of differing MIC. Bimodal distributions of MIC for ampicillin and penicillin were found in Staph. aureus isolates from or-ganic herds, suggesting that isolates with a higher MIC are a natural part of the bacterial population of the bovine mammary gland, or that isolates with higher MIC have persisted within these organic herds from a time when antimicrobials had been used
* β-lactam antimicrobials are the most commonly used DCT products in New Zealand, with 25% containing ampicillin, 61% containing cloxacillin, and 13% containing cephalonium, by mass (ACVM, 2019). Resistance to β-lactam antimicrobials may arise either through production of β-lactamases or via mutations of the penicillin-binding proteins of the bacterial cell wall (Walsh, 2000)
* The objective of the current study was to compare the distribution of MIC for the common mastitis pathogens, CNS, Staph. aureus, and streptococci from cows that received DCT containing primarily cephalonium or ampicillin-cloxacillin or cows from organic herds that had not used any antimicrobial therapy in pasture-based New Zealand herds
* 26 herds, 7 organic (not used AB in last three years) – certified organic for median of 12 years, range 7-19
* The estimated percentage of cows treated in each herd was determined by dividing the total number of cows in the herd (derived from the maximum number of animals present at herd testing or pregnancy diagnosis) by the estimated number treated. It was assumed that where “blanket” DCT was used, approximately 75 to 80% of the herd would be treated, as approximately 20% of cows in New Zealand dairy herds are culled each year. Herds were then selected on the basis that >50% of cows were treated in each of the 3 previous years with 1 DCT product. The DCT products were categorized into those containing cloxacillin benzathine (500 or 600 mg), cloxacillin (500–600 mg) and ampicillin (250–300 mg) combination products, or cephalonium (250 mg). The total mass was then divided by the number of cows in the herd and the assumed mass of an average New Zealand dairy cow (450 kg) to determine the total mass of antimicrobials used per kilogram of liveweight per year for each herd. The mass of each class of antimicrobial per kilogram of liveweight per year was also calculated. Cows were selected from each en-rolled herd that had had at least 1 lactation, had been treated with DCT (in those herds using DCT), had not been treated with any other antimicrobial within 30 d before sample collection (to maximize the chance of isolating bacteria when sampled), and had an individual SCC of >200,000 cells/mL at the most recent production recording. Staphylococcus aureus were defined as gram-positive, catalase- and coagulase-positive cocci
* MIC determination using commercially available broth microdilution plates (CMV1AMAF; Thermo Scientific), which included 10 antimicrobials (Table 1). The MIC was determined fol-lowing the Clinical and Laboratory Standards Institute standards. For analysis, MIC at or below the lowest concentration tested were recoded as the lowest MIC, and isolates that were not inhibited by the highest concentration tested were reported as a value twice the highest concentration tested
* For the 11 herds that had used cloxacillin-ampicillin DCT, a total of 250 isolates of Staph. aureus, CNS, or streptococci were obtained; of these, 247 were selected for MIC determination. For the 8 herds that used cephalonium DCT, and from the 7 organic herds, there were 566 and 469 eligible isolates, respectively. A random selection of these isolates was chosen from each herd type for MIC determination, such that the number of each species was 110% of the number of isolates from herds that had used cloxacillin-ampicillin DCT
* The distribution of MIC for isolates of CNS, Staph. aureus, and Strep. uberis were compared between iso-lates from herds that used cephalonium or cloxacillin-ampicillin DCT and from organic herds, for each an-timicrobial, using binomial or multinomial logistic re-gression. The MIC values for each antimicrobial within each bacterial class were visually assessed and collapsed into categories such that there were 10 or more values within each MIC category. For some antimicrobial-bac-teria combinations, the great majority of MIC values were within 1 category such that no further analysis was undertaken with these antimicrobial-bacterial com-binations. The logistic regression models included DCT type as the main effect and used robust standard errors to account for clustering of isolates within herd
* The two farm types using AB didn’t differ in AB usage, except for the dry treatment used. The mean total antimicrobial usage per year (as ex-pressed on a mg/kg of live weight basis) in the 3 yr before the study did not differ between farms that used cephalonium or cloxacillin-ampicillin DCT, nor did the usage of any specific class of antimicrobials, except for those antimicrobials used for DCT
* The MIC50and MIC90 for different antimicrobials did not differ by more than one dilution between isolates from herds using different DCT or from organic herds, except for the MIC90 for **ampicillin and penicillin, which were lower in isolates from organic herds than from herds using DCT**
* CNS (240)
  + At the univariate level, the proportion of penicillin-resistant CNS isolates were higher in herds that used cephalonium DCT (42/82; 51%) and from herds that used cloxacillin-ampicillin DCT (22/74; 30%) than from organic herds (14/84; 17%) (P < 0.001)
  + From the final multivariable logistic regression mod-el, the estimated marginal mean (± SE) proportion of penicillin-resistant CNS isolates was greater from herds that used cephalonium DCT (0.50 ± 0.07) than from herds that used cloxacillin /ampicillin DCT (0.31 ± 0.06) and from organic herds (0.17 ± 0.05)
  + The ceftiofur MIC results were categorized as 0.5, 1, or 2 μg/mL; the erythromycin MIC were categorized as 0.25, 0.5 or ≥1 μg/mL; and the sulfadimethoxine MIC were categorized as 32, 64 to 256, and 512 μg/mL, respectively, for subsequent multinomial logistic regres-sion. There were more ceftiofur MIC of 0.5 mg/mL and fewer with an MIC of 2 mg/mL from the organic iso-lates than from isolates from herds using DCT (Figure 3). More isolates from herds using cloxacillin-ampicillin DCT had an MIC ≥1 mg/mL for erythromycin than isolates from the other herds (Figure 3). There was no significant difference in the distributions of MIC among isolates from different herd types for sulfadimethoxine (Figure 3
* Aureus (320)
  + The MIC50 for ampicillin and penicillin were greater by more than 1 dilution for isolates of Staph. aureusfrom herds that used cephalonium DCT compared with herds that used cloxacillin-ampicillin DCT or from or-ganic herds. The MIC90 for these antimicrobials for iso-lates from cephalonium DCT herds, and from organic
  + At the univariate level, the proportion of penicillin-resistant Staph. aureus isolates was higher in herds that used cephalonium DCT (76/111; 68.5%) than in herds that used cloxacillin-ampicillin DCT (4/99; 4.0%) or organic herds (32/110; 29.1%; P < 0.001).
  + The estimated marginal mean (± SE) proportions of penicillin-resistant isolates did not differ between iso-lates from herds that used cephalonium (0.40 ± 0.14) or cloxacillin-ampicillin (0.14 ± 0.08) DCT or from organic herds (0.34 ± 0.11; P = 0.30). The likelihood ratio test indicated a significantly better fit for the multilevel model than for a logistic regression model (P < 0.001), indicating significant clustering of isolate within herd.The ceftiofur MIC results were categorized as 0.5, 1, or 2 μg/mL, the erythromycin MIC was categorized as 0.25 or ≥0.5 μg/mL, and the sulfadimethoxine MIC were categorized as 32, 64 to 256, and 512 μg/mL, respectively, for subsequent multinomial or binomial logistic regression. There were fewer ceftiofur MIC of 1 μg/mL in the organic isolates compared with iso-lates from herds using DCT (Figure 5), and there were no other differences among DCT groups in the other MIC categories. More isolates from organic herds had an MIC for sulfadimethoxine of 32 μg/mL compared with isolates from herds using DCT
* Strep dys (50)
  + When isolates that were defined as intermediate and resistant for tetracycline using CLSI breakpoints were combined, the proportion of isolates that were not susceptible tended to be lower in herds that used cephalonium DCT (6/17; 35%) than herds that used cloxacillin-ampicillin DCT (11/16; 69%) or organic herds (12/17; 71%; P = 0.07). The MIC50 and MIC90 for different antimicrobials did not differ by more than 1 dilution between Strep. dysgalactiae isolates from herds using different DCT or from organic herds, except the MIC90 for erythromy-cin, which was higher in isolates from herds that used cephalonium DCT than herds that used cloxacillin-ampicillin DCT or organic herds
* Strep uberis (184)
  + The MIC50 and MIC90 for different antimicrobials did not differ by more than 1 dilution between Strep. uberis isolates from herds us-ing different DCT or from organic herds (Table 12). The ceftiofur MIC results were categorized as 0.5 and ≥1 μg/mL, and the penicillin MIC were categorized as 0.12 or ≥0.25 μg/mL, respectively, for subsequent binomial logistic regression. There were fewer ceftiofur MIC results ≥1 μg/mL and fewer penicillin MIC ≥0.25 μg/mL, respectively, in the organic isolates compared with isolates from herds using DCT
* This study evaluated the MIC of CNS, Staph. aureus, Strep. dysgalactiae, and Strep. uberis isolated from milk of dairy cows with elevated SCC, from organic herds, and herds that predominantly used either cephalonium or cloxacillin/ampicillin–based DCT. Generally, the prevalence of nonsusceptible isolates as defined from CLSI breakpoints was low other than for ampicillin, penicillin, and oxacillin for CNS; ampicillin, and penicil-lin for Staph. aureus; tetracycline for Strep. dysgalactiae, and penicillin for Strep. uberis. For most antimicrobials, the MIC50 and MIC90 were similar among isolates from organic herds and from herds that used cephalonium and ampicillin-cloxacillin DCT. However, there was evidence of differing MIC distributions for some anti-microbials, suggesting that exposure to antimicrobials was associated with increased MIC for some bacteria. Bimodal distributions of MIC for penicillin were found among Staph. aureus isolates from organic farms. This suggests either that resistant isolates had persisted on those farms from the time when they were using anti-microbials, that resistant Staph. aureus isolates were imported, or that Staph. aureus isolates with high MIC are found in populations not exposed to antimicrobials
* For the CNS isolates in the current study, the MIC90for ampicillin and penicillin were lower in isolates from organic herds than from herds using cephalonium or cloxacillin-ampicillin DCT. In addition, CNS isolates from organic herds were more likely to have an MIC in the lowest category tested (0.5 μg/mL) and less likely to have an isolate in the higher MIC categories (≥2 μg/mL) for ceftiofur
* The MIC90 for ampicillin and penicillin was lower in Staph. aureus isolates from herds using ampicillin-cloxacillin DCT than from herds that had used cepha lonium DCT or that were organic. Similarly, at the bivariate level, the MIC distributions for these anti-microbials differed between isolates from herds using ampicillin-cloxacillin or cephalonium DCT, but not be-tween isolates from herds that used cephalonium DCT compared with isolates from organic herds. Previous studies have found that the risk of being above MIC90only differed between Staph. aureus isolates from bulk tank milk from organic or conventional dairy farms for ciprofloxacin (in Wisconsin herds) and albamycin (in Danish herds) among 15 antimicrobials tested (Sato et al., 2004), and there was no difference between Scandi-navian conventional or organic herds in the percentage of herds in which penicillin-resistant Staph. aureus was found, or the proportion of cows with Staph. aureusisolates that were penicillin-resistant (Bennedsgaard et al., 2006; Garmo et al., 2010). Similarly, a Swiss study comparing 60 organic and 60 conventional dairy farms found no difference in the proportion of gram-positive mastitis isolates that were resistant, as defined by CLSI breakpoints (Roesch et al., 2006
* although there were differences in the MIC distributions among Strep. uberis isolates from herds that had used DCT and from organic herds, in almost all cases, this was due to a higher proportion of isolates from organic herds being present in the lowest concentration tested. Where the MIC are below the clinical breakpoint, it is currently unclear as to whether these variations in MIC distribution are clinically significant
* conversely, no differences between organic and DCT-using farms in MIC distributions were detected for erythromycin, penicillin-novobiocin, or for oxacillin, the class representative for cloxacillin. In the current study, similar proportions of Strep. Uberis isolates had an elevated MIC for oxacillin among organic herds and those using DCT. This suggests that exposure to antimicrobials does not necessarily explain the presence of elevated MIC for oxacillin among Strep. uberis isolates
* Other studies have demonstrated that phenotypic and genotypic resistance against tetracyclines and macrolides may be found without exposure to those classes of antimicrobials (Dunlop et al., 1998; Holman and Chénier, 2013; Agga et al., 2014). Associations were found between the amounts of specific antimicrobials used for treatment and the probability of resistant or multidrug-resistant Staph. aureus and CNS (Saini et al., 2012; Nobrega et al., 2018).
* prevalence of antimicrobial resistance on a specific farm may not be correlated with antimicrobial usage on that farm. For example, animal movements may increase the risk of introducing resistant isolates or genes, and use of heavy metals or disinfectants may result in cross selection for antimicrobial resistance among bovine isolates (Davies and Wales, 2019). Sublethal exposure to some disinfectants such as chlorhexidine, widely used in the dairy industry as teat disinfectant, may increase the frequency of conjugated transfer of antimicrobial resistance genes (Jutkina et al., 2018)
* In the context of the current study, the presence of antimicrobial resistance on farms that had been organic for at least 3 yr, and in some cases up to 19 yr, demonstrated that ceasing use of antimicrobials does not necessarily result in elimination of antimicrobial resistance.
* We conclude that, aside from ampicillin and penicillin resistance among CNS and Staph. aureus isolates and tetracycline resistance among Strep. Dysgalactiae isolates, antimicrobial resistance to commonly used antimicrobials among mastitis pathogens in New Zealand. However, the MIC for some antimicrobials was higher for isolates from herds using DCT compared with organic herds. This suggests that some selection pressure is occurring in herds using antimicrobials relative to organic herds. It should be noted that the differences in MIC distributions were relatively small. Even though data supporting clinical breakpoints is currently lacking for many bacteria-antimicrobial combinations, the MIC were such that antimicrobials likely remain clinically effective. It should also be noted that antimicrobial resistance was present among some iso-lates from organic herds, suggesting factors other than current antimicrobial use may be contributing to presence of resistant isolates within herds. The duration of persistence of antimicrobial resistance, once present in herd, and the risk of importation of resistant isolates, remains unknown and requires further study

***Short Communication: Prevalence and antibiotic resistance of mastitis pathogens isolated from dairy herds transitioning to organic management***

*Park 2012*

* Case report of two farms transitioning over three years from conventional to organic
* American organic farms (no AB at all)
* Changes in udder health and antibiotic resistance of mastitis pathogens isolated from dairies upon conversion from conventional to organic management over a 3-year period was studied. Coagulase-negative staphylococci (CNS) were the most prevalent mastitis pathogens isolated. CNS were significantly less resistant to β-lactam antibiotics when isolated from milk after the herd transitioned to organic management. Cessation of the use of antimicrobial therapies in dairies in combination with organic management could lead to a reduction in the antimicrobial resistance of mastitis pathogens.
* The purpose of this study was to determine changes in intramammary infection (IMI) prevalence and antimicrobial resistance in mastitis pathogens isolated at the end of lactation and at parturition. Two herds in their last year of conventional dairy production, during their transition year, and during their first year of organic production, were studied. During the conventional period, cows with clinical mastitis received a commercial lactating cow intramammary product with pirlimycin hydrochloride (Pfizer Animal Health, USA). Non-lactating cow intramammary products of cephapirin sodium or dihydrostreptomycin sulfate and penicillin G procaine or novobiocin sodium and penicillin G procaine (Pfizer Animal Health, USA) were used. Composite milk samples were aseptically collected from cows at the end of lactation and within 1 day of parturition, and from cows with clinical mastitis, during all three years
* Antibiotic resistance testing was as described [2]. Interpretation of zone diameter was carried out according to Clinical and Laboratory Standard Institute (CLSI) guidelines. ampicillin, cephalothin, cloxacillin, erythromycin, gentamicin, lincomycin, neomycin, nitorfurantoin, novobiocin, penicillin, streptomycin, tetracycline
* There was a significant increase in zone diameter around the ampicillin, cephalothin, cloxacillin, and penicillin discs for CNS according to the period (conventional vs. transition vs. organic phase) (p < 0.05, Table 2). However, there were no significant changes in the sensitivity patterns of the antibiotics to Streptococcus spp. and S. aureus (Table 2). Antibiotic resistance of the coliforms was not analyzed due to the low infection rate. The percentage of CNS isolates deemed resistant to β-lactam antibiotics decreased from the conventional period compared to the organic period
* change in antibiotic resistance that occurs during the longitudinal transition from conventional to organic management. In this study, the decreased β-lactam resistance rate of CNS paralleled the discontinuation of the use of β-lactam antibiotics

***Letter to the Editor: Methicillin-Resistant Staphylococcus epidermidis in Organic Milk Production***

*Walther 2007*

* Case report of isolate from organic farm
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* **A dairy cow from a farm for organic milk production**, where the use of antibiotics is restricted, was diagnosed twice within 2 mo with **subclinical mastitis caused by methicillin-resistant Staphylococcus epidermidis**. The strains also displayed **resistance to chloramphenicol and harbored streptomycin- and trimethoprim-resistance** genes that remained silent in vitro. The **second isolate contained an additional aminoglycoside-resistance gene** indicating the potential acquisition of **resistance by horizontal gene transfer**.
* In organic dairy farming, the prophylactic use of antibiotics is not permitted. In cases of subclinical mastitis, antibiotics can only be given if previous homeopathic treatments have failed and if antibiotic resistance profiles of isolated pathogens have been determined.
* (Roesch et al., 2006). Six-teen and 18% of the cows from organic and conventional farms, respectively, harbored staphylococci displaying resistance to oxacillin, the indicator antibiotic used to predict the presence of the methicillin-resistance gene (mecA) in staphylococci. This gene, which mediates resistance to allβ-lactam antibiotics, was only detected in isolates from organic farms
* This finding shows that food-producing animals kept on organic farms may harbor multidrug-resistant staphylococci despite the limited use of antibiotics
* bacterial contamination of cows on the farm and the spread of bacteria with pathogenic potential to humans through the food chain. Organic milk destined for raw milk cheese production, as well as cheese made thereof, should be subject to careful bacteriological analysis and should not contain bacteria harboring transferable antibiotic resistance genes

***Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows***

*Rajala-Schultz 2004*

* Comparison of different parities of animal in a conventional farm (US)
* The purpose of this study was to describe and compare antimicrobial susceptibility of mastitis pathogens isolated at calving from first lactation and older cows. A total of 202 bacteria were isolated from intramammary infections (IMIs) within 3 days after calving over a 16-month study period in OH. Of these IMIs, 78% were caused by coagulase-negative staphylococci (CNS). Forty-four percent of them were resistant to at least one antibiotic. Most resistance was observed against penicillin, 39% of the isolates from older cows and 26% from first lactation cows being resistant to penicillin (P > 0.05). Also MIC90 for penicillin was higher among isolates from older cows. On the other hand, resistance to tetracycline was more common and MIC90 higher among isolates from first lactation cows than from older cows. Differences in the proportions of resistant isolates between first lactation and older cows were not statistically significant, though (? For peniciilin?). The resistance patterns of the CNS isolated during the study are concordant with antimicrobial usage in the study herd
* Microflora of most older cows has experienced direct selective pressure from antibiotics through dry cow and potentially other mastitis therapy, whereas heifers have typically not been treated with any mastitis preparations before their first calving. The objective of this study was to describe and compare susceptibility patterns of mastitis pathogens isolated at calving from 1st lactation and older cows … The persistent nature of dry treatment preparations could thus provide a strong selective pressure for the acquisition and maintenance of drug resistance genes
* Staphylococcal isolates were examined for coagulase production using the tube test and rabbit plasma. Coagulase tests were incubated for a total of 24 h and read at 4 and 24 h. Staphylococcal isolates were further tested using the **API Staph to determine species**
* semi-automated Sensititre system following manufacturer’s guidelines. A commercially available micro dilution panel, designed for mastitis pathogens, was used to determine the minimum inhibitory concentration (MIC) of 10 different antimicrobial drugs … isolates categorized as susceptible, intermediate or resistant according to methods and criteria described by National Committee for Clinical Laboratory Standards (NCCLS, 2002)
* Penicillin, Ampicillin, Oxacillin, Cephalothin, Ceftiofur, Penicillin + novobiocin, Erythromycin, Pirlimycin, Tetracycline, Sulfadimethoxine
* The lowest concentrations inhibiting 50% (MIC50) and 90% (MIC90) of the isolates were determined. Antimicrobial dry cow therapy has been routinely used in all cows in the study herd for years. Dry cow preparations containing either penicillin– dihydrostreptomycin or cephalothin are normally used in alternate years. Penicillin and ceftiofur are the most commonly used systemic antimicrobials in lactating cows in the herd. Calves are fed milk replacer which contains tetracycline and neomycin
* We did not, however, observe any statistically significant differences in the proportion of bacteria resistant to the 10 antimicrobials tested between 1st lactation and older cows. The biggest observed difference was in the amount of resistance against penicillin: proportion of resistant bacteria was about 13% higher among the multiparous than in primiparous cows, but the difference was not statistically significant due to a relatively small sample size and thus low statistical power.
* Isolates with MIC in the intermediate range were categorized as resistant for data analysis. The proportion of bacteria resistant to each antibiotic was calculated. Also, the proportion of bacteria resistant to at least one of the 10 tested antibiotics and resistant to two or more antibiotics were calculated
* The majority of the isolates (158 of 202, 78.2%) were coagulase-negative staphylococci (CNS), S. chromogenes being the most prevalent species (105/158). Other commonly isolated CNS species were S. hyicus (n = 16), S. simulans (n = 16), and S. epidermidis (n = 7). A CNS was isolated from all of the 48 1st lactation cows with IMI and in 9 of these cows at least one other pathogen was isolated either from the same or another quarter of the cow.
* One hundred and eighty isolates (180) were successfully revived for susceptibility testing, 139 of those being CNS. The MICs and proportion of resistant bacteria for CNS are presented in Table 3. Of the tested CNS isolates, 43.9% (n = 61) were resistant to at least one antibiotic. Most resistance was observed against penicillin (44/139, 31.7% of the isolates resistant, MIC > 0.25), followed by resistance to ampicillin (17/139, 12.2% of the isolates, MIC > 0.5), sulfadimethoxine (17/139, 12.2% of the isolates, MIC > 256) and tetracycline (16/139, 11.5% of the isolates, MIC > 8.0). Some resistance was also observed against erythromycin (7.9%) and pirlimycin (5.0%). Altogether 28 of the 139 isolates (20.1%) were resistant to at least two antibiotics (13 of them from primiparous and 15 from multiparous cows) and 11 (7.9%) were multiresistant (resistant to three or more antibiotics). Of the multiresistant CNS strains, five were isolated from primiparous and six from multiparous cows. All isolates resistant to ampicillin, except for one, were resistant also to penicillin. Also, seven of the eight isolates that were resistant to penicillin–novobiocin combination (MIC > 4.0/8.0) and all four isolates resistant to oxacillin (MIC > 4.0) were resistant to penicillin. Two isolates were categorized as resistant both to cephalothin (MIC > 16) and ceftiofur (MIC > 2.0). These isolates were resistant to all other beta-lactam antibiotics as well. They were isolated from first lactation cows. Resistance patterns appeared not to vary between different CNS species.
* The largest difference in the proportion of resistant CNS between primiparous and multiparous cows was against penicillin (26.5% resistant to penicillin in primiparous and 39.3% in multiparous). In accordance with this, MIC90 for penicillin was also higher in pathogens from older cows than younger cows
* With all the other antimicrobials, the difference in the proportion of organisms being resistant among young and older cows was less than 5%, except with erythromycin where the difference was 8%, pathogens from older cows exhibiting more resistance than younger cows. None of the differences were statistically significant (P-value with penicillin resistance was 0.11). Even when controlling for the interdependency of the isolates in the analysis, parity was not a significant explanatory variable
* An interesting observation from this study was that CNS isolates from primiparous cows had higher MIC90’s for tetracycline and sulfadimethoxine than multiparous cows and that also the proportion of isolates resistant to tetracycline was higher in first lactation than in older cows. The calf milk replacer used in the herd contains tetracycline and neomycin which could be an explanation for the above observation. We do not have an explanation why MIC90 for sulfadimethoxine was higher among young than older cows as it is not typically used in treating young calves or growing heifers. Sulfa resistance, however, is often carried along with tetracycline resistance in plasmids, which could be the reason for this (Tanal et al., 1976; Prescott, 2000)

***Antimicrobial resistance in beef and dairy cattle production***

*Call 2008*

* Review article: summary of observational studies, puts forth hypothesis about why AMR maintained in absence of AB usage pressure
* *“there are multi-factorial events and pressures that influence AMR bacterial populations in cattle production systems”*
* Observational studies of cattle production systems usually find that cattle from conventional dairies harbor a higher prevalence of antimicrobial resistant (AMR) enteric bacteria compared to organic dairies or beef-cow operations; given that dairies usually use more antimicrobials, this result is not unexpected. Experimental studies have usually verified that application of antimicrobials leads to at least a transient expansion of AMR bacterial populations in treated cattle. Nevertheless, on dairy farms the majority of antibiotics are used to treat mastitis and yet AMR remains relatively low in mastitis pathogens. Other studies have shown no correlation between antimicrobial use and prevalence of AMR bacteria including documented cases where the prevalence of AMR bacteria is non-responsive to antimicrobial applications or remains relatively high in the absence of antimicrobial use or any other obvious selective pressures. Thus, there are multi-factorial events and pressures that influence AMR bacterial populations in cattle production systems. We introduce a heuristic model that illustrates how repeated antimicrobial selection pressure can increase the probability of genetic linkage between AMR genes and niche- or growth-specific fitness traits. This linkage allows persistence of AMR bacteria at the herd level because subpopulations of AMR bacteria are able to reside long-term within the host animals even in the absence of antimicrobial selection pressure. This model highlights the need for multiple approaches to manage herd health so that the total amount of antimicrobials is limited in a manner that meets animal welfare and public health needs while reducing costs for producers and consumers over the long-term
* Why do we care about AMR in pathogens on dairy systems?
  + Antimicrobial resistance (AMR) is clearly a concern in cattle production systems where AMR pathogens can contribute to increased morbidity and mortality of livestock with commensurate increases in production expenses for livestock producers (Mathew et al., 2007). From a public health perspective there is potential for AMR pathogens and commensal organisms to disseminate to humans via direct contact with animals (Price et al., 2007) or via the food chain (van den Bogaard and Stobberingh, 2000; Silbergeld et al., 2008)
* most obvious selection pressure for AMR is application of antimicrobials for treatment (e.g. mastitis, lameness, respiratory illness, and scours) and for prophylactic health benefits and production gains (e.g. medicated milk replacer)
* **These practices can promote AMR by two potential mechanisms:** they permit AMR bacterial populations to expand in numbers by providing a competitive advantage for resistant strains, and they permit resistance genes to disseminate successfully to new bacterial hosts if these genes are harbored on horizontally transmissible elements such as plasmids and conjugative transposons
* Summary of fecal AMR work:
  + Published comparisons of these systems have targeted generic Escherichia coli (Sato et al., 2005), Shiga-toxin producing E. coli (STEC) (Cho et al., 2007), Salmonella (Ray et al., 2006), and Campylobacter (Sato et al., 2004; Halbert et al., 2006). For fecal/enteric pathogens, findings have been variable. Ray et al. (2006) found that conventional dairies were more likely to yield Salmonella with resistance to streptomycin or sulfonamides. Sato et al. (2005) compared conventional and organic systems and found that the former had a significantly higher prevalence of AMR for fecal E. coli across multiple drug classes (ampicillin, tetracycline, sulfonamides, kanamycin, gentamicin, chloramphenicol and tetracycline), but no differences were detected for 10 other antimicrobials. Two studies that examined Campylobacter found no significant differences in AMR between production systems, although Halbert et al. (2006) found significantly higher prevalence of resistance to tetracycline in conventional farm isolates. Most of these studies were limited by herd number, particularly for organic dairies, and most involved only a single sampling date from which prevalence of resistance was calculated … younger animals are more likely to have resistant organisms in their feces when compared to older animals (Khachatryan et al., 2004; Sato et al., 2005; Cho et al., 2007)
  + while antimicrobial use can result in a higher prevalence of AMR fecal bacteria, the effect is often transient: Berge et al. (2005a), (Lowrance et al., 2007), (Stabler et al., 1982), Langford et al. (2003)
  + fecal bacteria may behave differently – transmission between species may be different (species vary in their behavior) and gut flora spp. generally different from gram positive mastitis pathogens
* Why AMR maintained?
  + Although there seems to be a consistent association between organic management and lower prevalence of AMR bacteria, resistant bacteria persist on organic farms even after years of antimicrobial-free management, suggesting that factors other than antimicrobial use play an important role in long-term persistence
  + After analyzing the phylogenetic groupings of E. coli isolates from the organic/conventional comparison (Sato et al., 2005), Walk et al. (2007) concluded that the overabundance of ampicillin-resistant populations on conventional dairies was a consequence of antimicrobial use, but that tetracycline resistance genetic determinants had established a steady-state and that their presence was unrelated to antimicrobial usage.
  + Ampicillin resistance among control group isolates (isolates from calves fed no antimicrobials) increased during the latter part of the study due to an evident clonal expansion of an environmental strain that outcompeted other strains of E. coli, as detected by pulsed-field gel electrophoresis (PFGE) patterns (Alexander et al., 2008). This suggests that fitness traits other than AMR can play an important role in the emergence and dissemination of AMR bacteria in food animals (see below).
  + Co-selection of AMR traits is an obvious case whereby AMR traits are genetically linked so that selection of one antibiotic resistance trait maintains the unrelated AMR trait (Borgen et al., 2002; Chen et al., 2008). Similar genetic linkages have been described for heavy metal resistance (Liebert et al., 1999; Hasman and Aarestrup, 2002; Hasman et al., 2006). In these cases, co-selection of AMR traits relies on a negative selection event (antimicrobial or toxin exposure), but there are multiple reports that AMR bacteria can persist in the absence of obvious negative selection pressures
  + Group of papers exploring this question:
    - Khachatryan et al. (2004, 2006a, b, 2008) examined this question with a focus on commensal E. coli resistant to streptomycin, sulfonamide and tetracycline (SSuT) in dairy calves
      * Was it direct antimicrobial selection pressure maintaining a high prevalence of SSuT strains? No - a simple clinical trial showed that addition or removal of oxytetracycline from the diet had no effect on the prevalence of SSuT strains over the shortterm (about 3 months)
      * Do SSuT traits themselves provide a secondary but unrecognized advantage to these strains? No- To test this hypothesis, Khachatryan et al. (2006b) generated null mutants for the SSuT traits (‘exSSuT’ strains) and demonstrated that, on average, these strains retained their competitive fitness advantage over pan-susceptible strains both in vitro and in vivo. Thus, the genes conferring the SSuT phenotype did not appear to provide any secondary fitness advantages
      * They had a competitive edge in the particular environment of the gut of these dairy calves fed this particular milk replacer – with or without oxytet. The SSuT strains predominated when calves fed milk replacer of dried milk, vitamin A and D (regardless of AB addition). A subsequent trial showed that reintroducing the milk supplement with or without oxytetracycline nearly doubled the prevalence of SSuT strains over animals receiving no supplement (Khachatryan et al., 2006a, 2008)
      * a dietary supplement was either directly or indirectly favoring strains of E. coli that harbored the SSuT resistance element. This particular linkage example does not explain persistence of other resistance traits in these dairy calf isolates, but it is illustrative of positive selection events that are challenging to detect and interpret in production environments
      * Singer et al. (2006) provide an excellent review of the complex array of factors across broader spatial scales that can decouple associations between antimicrobial exposure and AMR, and one potentially important factor in this pattern is coupling of AMR traits with other **niche-specific, selectively advantageous traits. … locally selective fitness traits**
    - AMR … Subsidence is expected if the AMR traits themselves afford a fitness cost in the absence of selection pressure. **OR** If fitness cost is neutral, we would still expect eventual displacement in the face of natural turnover of clonal types at the level of individual animals ( Jenkins et al., 2003). **OR** In other cases, there is no response to antimicrobial selection pressure or only a limited response and the prevalence of AMR bacteria remains high in the absence of obvious selection factors (Khachatryan et al., 2004). **OR** Another possibility is that antimicrobial traits become, on occasion, linked to other traits that offer niche-specific selective advantages
    - Linkage leads to better survival, but there is nothing to suppress competing flora that do not harbor the better fitness trait. Positively selected traits are much harder to detect empirically than negatively selected traits
    - *“A heuristic model illustrating how antimicrobial selection pressure leads to transient expansion of AMR subpopulations within individual animals. Over time these antimicrobial induced population expansions abate and the relative proportion of AMR subpopulations decline. Expanded populations also increase the likelihood of a genetic event whereby an AMR gene is linked to some other trait that confers a niche-specific fitness advantage in the host animal. When this latter event occurs, there is a long-term relative increase in the baseline prevalence of the AMR subpopulation that harbors this selective linkage.”*
* Good intro info for me – “history” of mastitis treatments
  + Mastitis is the most common condition that justifies use of antimicrobials on dairy operations (Zwald et al., 2004; Sawant et al., 2005; Raymond et al., 2006; Pol and Ruegg, 2007b). Mastitis is caused by a variety of Gram-positive and Gram-negative organisms (Erskine et al., 2002) and intramammary infusion of penicillin was the first antimicrobial treatment for this condition; in the US this practice dates from the mid-1940s (Bryan, 1947) to the present (Raymond et al., 2006). Consequently, from a ‘mass action’ perspective we would predict AMR to penicillin to be ‘high’ and this appears to be the case relative to other drugs, although penicillin susceptibility is still very common.

***Risk Factors for the Occurrence of Methicillin-Resistant Staphylococcus aureus in Dairy Herds: An Update***

*Schnitt 2020*

* Review article: MRSA in dairy systems
* Methicillin-resistant coagulase-negative staphylococci (MR-CoNS) were repeatedly isolated from dairy farms. This is an important issue since MR-CoNS may transfer resistance genes to S. aureus
* In S. aureus, methicillin resistance is mediated by a mecA or mecC- gene. This gene is located on a mobile genetic element called ‘‘staphylococcal cassette chromosome mec’’ (SCCmec). The gene is responsible for the production of an altered penicillin-binding protein 2a (PBP2a). The PBP2a has a lower affinity for b-lactam antimicrobials than the normal PBP. Thus, mecA-/mecC-positive staphylococci are resistant to most b-lactam antibiotics

***Comparison of Prevalence and Antimicrobial Susceptibilities of Campylobacter spp. Isolates from Organic and Conventional Dairy Herds in Wisconsin***

*Sato 2004*

* Head to head comparison (org. vs. conventional in same study)
* FECAL ISOLATES
* American organic farms (no AB at all)
* The prevalence and antimicrobial susceptibilities of Campylobacter spp. isolates from bovine feces were compared between organic and conventional dairy herds. Thirty organic dairy herds, where antimicrobials are rarely used for calves and never used for cows, were compared with 30 neighboring conventional dairy farms, where antimicrobials were routinely used for animals for all ages. Fecal specimens from 10 cows and 10 calves on 120 farm visits yielded 332 Campylobacter isolates. The prevalence of Campylobacter spp. in organic and conventional farms was 26.7 and 29.1%, and the prevalence was not statistically different between the two types of farms
* The gradient disk diffusion MIC method (Etest) was used for testing susceptibility to four antimicrobial agents: ciprofloxacin, gentamicin, erythromycin, and tetracycline. Two isolates were resistant to ciprofloxacin, and none of isolates was resistant to gentamicin or erythromycin. Resistance to tetracycline was 45% (148 of 332 isolates).
* We saw no evidence that restriction of antimicrobial use on dairy farms was associated with prevalence of resistance to ciprofloxacin, gentamicin, erythromycin, and tetracycline
* Campy considered commensal in cattle
* All organic farms were certified by an approved certification agency as not using antimicrobials for cows for at least 3 years (mean 8.0 years) before the start of our study. For each organic farm selected, the nearest conventional dairy farmer (in sequence of geographical proximity) was asked to serve as a control farm.
* 26 of the 30 conventional dairy herds, cows routinely received antimicrobial infusions into the udder at the cessation of each lactation cycle (“dry-cow treatment”). Cephapirin or penicillin was used most for this purpose. Eighteen conventional dairy producers reported using infusion of antimicrobials into the udder for the treatment of clinical mastitis. For severe cases of clinical mastitis, eight conventional dairy producers used systemic antimicrobials
* It is known that C. jejuni and C. coli have different susceptibility profiles (11). However, resistance traits are known to be readily transferred among species of Campylobacter (32), so a separate analysis for each species of Campylobacter was inadvisable given the ecological nature of our objectives regarding the use of Campylobacter spp. as an indicator of antimicrobial selective pressure on the entire bacterial community
* In our study, any bias due to the testing procedure should not have affected our comparison between organic and conventional farms. Any such systematic error or bias would have been a nondifferential misclassification bias that would have equally affected the organic and conventional farms (26). However, direct comparisons of MICs obtained from different methods should be interpreted with caution

***Antimicrobial Susceptibility of Salmonella from Organic and Conventional Dairy Farms***

*Ray 2006*

* Head to head comparison (org. vs. conventional in same study)
* FECAL ISOLATES
* American organic farms (no AB at all)
* The objective of this study was to compare antimicrobial susceptibility of Salmonella isolated from conventional and organic dairy farms in the Midwest and Northeast United States. Environmental and fecal samples were collected from organic (n = 26) and conventional (n = 69) farms in Michigan, Minnesota, New York, and Wisconsin every 2 mo from August 2000 to October 2001.Salmonellaisolates (n = 1,243) were tested using a broth microdilution method for susceptibility to amoxicillin-clavulanic acid, ampicillin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole
* For most antimicrobial agents tested, susceptibility of Salmonella isolates was similar on organic and conventional herds when controlling for herd size and state. Conventional farms were more likely to have at least one Salmonella isolate resistant to streptomycin using logistic regression (odds ratio =7.5; 95% confidence interval = 1.7-55.4). Conventional farms were more likely to have Salmonella isolates with greater resistance to streptomycin (odds ratio = 5.4;95% confidence interval = 1.5−19.0) and sulfamethoxazole (odds ratio = 4.2; 95% confidence interval =1.2−14.1) using logistic proportional hazards models. Although not statistically significant, conventional farms tended to be more likely to have at least one Salmonella isolate resistant to 5 or more antimicrobial agents when compared with organic farms
* Criteria for eligibility of dairy herds included having at least 30 milking cows, having at least 90% of cows of Holstein breed, raising their own replacement cattle, keeping a herd record system with unique identification for each cow, and shipping milk year round. All organic farms enrolled in this study were under organic manage mentor were certified organic for at least 3 yr prior to enrollment. Although no national organic standards existed at the time of this study, rules among organic certifying agencies in this study were similar to current national organic standards (USDA, 1999
* Environmental and fecal samples were collected at approximately 2-mo intervals from August 2000 to October 2001 at 32 organic farms and 97 conventional farms in Michigan, Minnesota, New York, and Wisconsin. Fecal samples were taken from healthy cows and target cattle groups consisting of preweaned calves receiving milk or milk replacer, cows to be culled within14 d, cows within 14 d of calving, and sick cows. Sick cows were defined as cows designated as sick by farmworkers or a veterinarian within the previous week or cows having clinical signs of illness evident to farmor project workers on day of visit (except for localized reproductive tract or mammary infections). The number of fecal samples collected per herd and cattle group at each visit was based on herd size and calculated to provide similar herd-level sensitivity of Salmonella detection assuming the same prevalence for all herds. Environmental samples: calving pen floor, sick pen floor, calf pen or hutch floor, feedbunk of lactating cows, lagoon or manure storage area, and bird droppings from cattle housing or feed storage areas
* Streptomycin was the only anti-microbial agent with a significant association between farm type and proportion of farms with resistance. Conventional farms were more likely to have at least one streptomycin resistant Salmonella isolate [odds ratio(OR) = 7.5; 95% confidence interval (CI) = 1.7−55.4]. Isolates from conventional farms were associated with higher streptomycin MIC than isolates from organic farms(OR = 5.4). A similar association was observed for sulfamethoxazole, with isolates from conventional farms exhibiting higher MIC than isolates from organic farms(OR = 4.2). n the multivariable analysis with state and farm type as categorical variables and herd size as a continuous variable, conventional farms tended to be more likely than organic farms to have at least one Salmonella isolate resistant to 5 or more antimicrobial drugs
* The most commonly reported antimicrobial agents used within the previous 60 d on conventional dairy farms were penicillins, cephalosporins, and tetracyclines (Zwald etal., 2004). Although resistance to these antimicrobial agents was observed among a high percentage of dairy herds, it is interesting to note that no significant difference in resistance to these individual antimicrobial agents was observed between organic and conventional dairy farms in our study
* Sulfonamide use was reported within the previous 60 d on 23.7% of conventional study farms compared with 0% of organic study farms. This might be a reason for the observed difference in increased resistance to sulfamethoxazole between Salmonella isolates from organic and conventional farms. Farm management type was not significantly associated with increased resistance to the other antimicrobial agents by logistic regression or logistic PH analysis; however, statistical power may not have been adequate for detecting a significant difference for some antimicrobial agents given our sample size of95 herds
* Before 1990, streptomycin was widely used to treat a variety of animal diseases. Streptomycin resistance could be due to an established resistance mechanism genetically linked to other beneficial genes on an integron or selected for by other antimicrobial agents utilizing the same resistance mechanism
* If recent antimicrobial drug use on individual farms were the sole factor associated with antimicrobial resistant Salmonella, we would expect to see greater differences between increased resistance and farm management type than what was observed. Our knowledge of antimicrobial use among the farms in our study is limited to herd-level, farmer-reported antimicrobial drug use so we were unable to examine the direct association between the amount of antimicrobial drug use and the antimicrobial resistance of Salmonella from these herds.
* Movement of animals, transport vehicles, wildlife, and personnel between herds may have facilitated the dispersion of antimicrobial resistant Salmonella among the dairy farms in our study. Our findings highlight the importance of examining factors other than antimicrobial use on individual farms, such as the spread of antimicrobial-resistant Salmonella between herds, when monitoring antimicrobial-resistant Salmonella on dairy farms
* Of the 14 antimicrobial agents tested, a significant association between increased resistance of Salmonella isolates from a dairy herd and farm management type was found only for streptomycin and sulfamethoxazole, with conventional farms harboring Salmonella isolates with more resistance

***Prevalence and risk factors for extended-spectrum β-lactamase or AmpC-producing Escherichia coli in organic dairy herds in the Netherlands***

*Santman-Berends 2017*

* Not a head to head (org. vs. conventional in same study)
* FECAL ISOLATES
* IN EUROPE, THESE FARMS WERE USING IMM ANTIBIOTICS even though they are organic
* Extended-spectrum β-lactamase and AmpC-produc-ing Escherichia coli (ESBL/AmpC) are an emerging problem and are hypothesized to be associated with antimicrobial use (AMU), and more specifically with the use of third- and fourth-generation cephalosporins. Whether ESBL/AmpC also occur in organic dairy herds, which have restricted AMU, is not known. Addi-tionally, it is unknown whether, in addition to restricted AMU, other factors in organic herd management are associated with ESBL/AmpC herd status. The aim of this study was to estimate the prevalence of ESBL/AmpC in organic dairy herds in the Netherlands. Sub-sequently, the relationships between the ESBL/AmpC herd status and AMU and between ESBL/AmpC herd status and farmers’ management were assessed in or-ganic dairy herds. For this study, 90 randomly selected, officially registered organic dairy herds were included. The ESBL/AmpC herd status was determined based on the bacteriological culture result of a slurry sample. From the data on antimicrobial supplies by the veterinarian, the animal daily defined dose of antimicrobials per farm per year (DDDAF) was calculated
* We found ESBL/AmpC in 12 of the 90 (13%; 95% confidence interval = 7–22%) slurry samples from organic dairy herds. The median DDDAF in organic dairy herds was 0.5, which was not significantly different between ESBL/AmpC-positive and unsuspected dairy herds. No association could be found between the use of different types of antimicrobials, such as third- and fourth-generation cephalosporins, and ESBL/AmpC herd status. The prevalence of ESBL/AmpC in organic dairy herds appeared lower than the prevalence in previous studies conducted in conventional dairy herds. Apparently, **ESBL/AmpC are also present in herds with low AMU; this indicates that other factors than AMU are also associated with ESBL/AmpC herd status**.
* These ESBL/AmpC-producing E. coli (ESBL/AmpC) have been demonstrated to be related to resistance against third- and fourth-generation cephalosporins in humans and animals, which are defined as critically important antimicrobials by the World Health Organization
* High prevalence of ESBL-producing E. coli in ani-mals have been demonstrated in many studies and vary between countries and animal species. In cattle, studies focused on ESBL/AmpC in veal, dairy, and beef cattle and showed prevalence ranging between 1 to 32.8% on an animal level and 35.4 to 86.7% on a herd level
* In European registered organic dairy herds, a restricted antimicrobial usage (AMU) policy is applied that prohibits AMU unless it is prescribed by a veterinarian and with a maximum frequency of 3 treatments per cow per year (European Union regulation 834/2007 and 889/2008; EC, 2007, 2008).
* As the use of this type of antibiotic is known to be associated with the ESBL/AmpC herd status, it is hypothesized that the ESBL/AmpC herd prevalence in organic dairy herds is lower than in conventional dairy herds
* All milking cows of the first 90 organic farmers that agreed to par-ticipate were included in the study and are described as herds in the remainder of this paper. These herds represent organic herds and have a low AMU compared with conventional herds. According to European Union regulation (EC, 2007, 2008), these herds are obliged to apply a very restrictive AMU policy
* Confirmed E. coli isolates were examined for ESBL or AmpC production by the combination disc diffusion test using cefotaxime and ceftazidime with and without clavulanic acid, according to CLSI guidelines (CLSI 2011), and cefoxitin
* The total DDDAF did not differ significantly between ESBL/AmpC-positive or -unsuspected organic dairy herds (both 0.53). The DDDAF of the application methods, DDDAF,oral, DDDAF,dry, and DDDAF,mast, was not significantly dif-ferent between ESBL/AmpC-positive and -unsuspected organic dairy herds either.
* Other mgmt. factors were important in multivariable model for presence of ESBL/Amp-C (being close to pig farm), parenteral treatment for mastitis, feeding milk replacer instead of colostrum,
* In a previous study evaluating the ESBL/AmpC prevalence in conventional Dutch dairy herds using the same sampling method in the same time period, 41% of the herds tested positive (Gonggrijp et al., 2016). Other studies also found ESBL/AmpC herd prevalence in cattle herds that were higher compared with those found in the organic herds in our study, ranging between 25 and 86.7%; Although individual differences exist, conventional herds generally have a higher AMU than organic herds.
* Although the ESBL/AmpC herd prevalence in the organic dairy herds in our study was low with 13%, it was not negligible; thus, the use of third- and fourth-generation cephalosporins apparently is not the only factor associated with the ESBL/AmpC status in dairy herds. … The variance explained by the final model was only 13%, which indicates other factors are likely associated with ESBL/AmpC herd status that were not included in our study. … adding in AMU did not help model so wasn’t even included