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describing the proportion of staphylococci from CON herds resistant to different antimicrobials taken from a previously unpublished survey by the authors (completed 6 years prior).3  Proportions of *S. aureus* isolates from ORG herds resistant to different antimicrobials were similar to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

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

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