***Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine milk samples***

**Sampimon 2009**

* ***Country***
  + Netherlands
* ***Species described***
  + 17 different CNS
* ***Num. isolates***
  + 170
* ***Clinical or subclinical***
  + Doesn’t specify
* ***overall findings*** 
  + Phenotypic resistance and detection of resistance genes other than blaZ were relatively rare in Staph. chromogenes. In Staph. epidermidis, phenotypic resistance to penicillin was more common than in other species and almost half of the Staph. epidermidis isolates carried multiple resistance genes
  + high prevalence of genotypic resistance, particularly carriage of mecA, or presence of multiple resistance genes, were primarily detected in species with relatively low prevalence, such as Staph. cohnii subsp. cohnii, Staph. equorum, Staph. fleurettii, or Staph. sciuri. Of most concern is the intermediate position occupied by Staph. epidermidis. It was the second most common CNS species in this study and carried multiple resistance genes in close to half of the isolates
  + Phenotypic findings:
    - Flueretti and epidermidis had highest resistance to penicillin; Phenotypic oxacillin resistance was most commonly found in Staph. fleurettii, Staphylococcus cohnii subsp. cohnii, and Staph. xylosus; Phenotypic resistance to ML antibiotics was most prevalent in Staph. cohnii subsp. Cohnii, followed by Staph. equorum, and Staph. epidermidis
    - In **Staph. chromogenes, phenotypic lincomycin or oxacillin resistance was significantly less common** than in other species resistant to lincomycin or oxacillin in Staph. chromogenes versus resistant to lincomycin and resistant to oxacillin in other species. In **Staph. epidermidis, phenotypic resistance to penicillin was significantly more common** than in other species (16 of 23 isolates (70%) resistant in Staph. epidermidis versus 35 of 147 isolates (24%) in other species; P < 0.0001). In **Staph. equorum**, both phenotypic **erythromycin and lincomycin resistance were overrepresent**ed (3 of 10 isolates (30%) and 6 of 10 isolates (60%), respectively, in Staph. equorum vs. 9 of 160 isolates (6%) and 36 of 160 (23%), respectively, in other species; P = 0.02 for both comparisons). In **Staph. xylosus, phenotypic oxacillin resistance was overrepresented** (12 of 15 isolates resistant in Staph. xylosus vs. 35 of 155 in other species; P < 0.001).
  + Genotypic findings:
    - Of the eight resistance genes tested, blaZ was found most frequently, followed by lnuA and mecA,
    - Among ML resistance genes, the RNA methylase genes ermA and ermB were not detected whereas ermC was detected in five isolates belonging to four CNS species
* ***Generally helpful info/stuff from other studies***
  + In CNS, resistance against penicillin, methicillin, lincosamides, or macrolides has been described. Antimicrobial resistance patterns may differ between CNS species; (Gentilini et al., 2002; Lüthje and Schwarz, 2006; MARAN, 2007; Sawant et al., 2009). CNS can carry a number of resistance genes, including the mecA gene that encodes methicillin resistance. Thus, CNS may act as a reservoir for evolution of resistance in Staphylococcus aureus (Mevius et al., 2005), or pose a direct human health hazard, as for example in the case of methicillin resistant Staphylococcus epidermidis (MRSE) (Walther and Perreten, 2007). Knowledge of CNS-species specific resistance patterns may contribute to species-specific treatment
  + blaZ in CNS tends to be chromosomally located 108 Chapter 6 whereas blaZ in Staph. aureus is predominantly plasmid borne (Olsen et al., 2006). Chromosomal and plasmid borne blaZ have largely separate phylogenetic histories, suggesting that transfer of blaZ between chromosomal and plasmid DNA is an extremely rare event (Olsen et al., 2006). Because bovine CNS primarily carry chromosomal blaZ, whereas blaZ in bovine and human Staph. aureus is primarily plasmid borne, it would be tempting to conclude that they do not represent a risk as potential source of blaZ genes for Staph. aureus. Indeed, it has been stated that Staph. aureus and CNS do not normally share the blaZ gene pool (Olsen et al., 2006). However, plasmid borne blaZ in bovine CNS does exist, and it is highly homologous 111 CNS and antimicrobial susceptibility to human plasmid borne blaZ (Olsen et al., 2006
  + A bigger concern than transfer of blaZ is transfer of mecA (Chambers, 1997). Staphylococcus aureus is thought to have acquired SCCmec, the cassette that includes mecA and its regulatory genes, on at least 20 occasions (Deurenberg et al., 2007). mecA-positive CNS are a potential reservoir for these elements (Hanssen and Ericson Sollid, 2006). Examples of likely transfer of mecA from CNS to Staph. aureus include the formation of MRSA in a patient during treatment through horizontal transfer of mecA DNA from an Staph. epidermidis strain (Wielders et al., 2001), and development of an MRSA strain in a neonatal ward due to horizontal transfer of an SCCmec element from methicillin resistant Staph. haemolyticus (Berglund and Söderquist, 2008).
  + the majority of ML genes in CNS are plasmid-borne
* ***speciation***
  + genetic
* ***type of resistance***
* phenotypic AND genetic
* ***Methodology***
  + Phenotypic = MIC from microbroth dilution method; penicillin (≥0.25 mg/l), oxacillin (≥0.5 mg/l), cephalotin (≥32 mg/l), erythromycin (≥8 mg/l), pirlimycin (≥4 mg/l), streptomycin (≥32 mg/l), and tetracycline (≥16 mg/l), lincomycin (≥8 mg/l), kanamycin (≥32 mg/l), and neomycin (≥32 mg/l); Oxacillin was included as indicator for methicillin resistance
  + Genotypic
    - penicillin/methicillin resistance, blaZ and mecA
    - macrolide and lincosamide (ML) resistance genes ermA, ermB, ermC, msrA, and lnuA
    - macrolide msrA, mphC

***CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis***

**Persson Waller 2011**

* ***Country***
  + Sweden
* ***Species described***
  + 14 different species
* ***Clinical or subclinical***
  + Both and differentiates
* ***Num. isolates***
  + 154
* ***overall findings*** 
  + b-Lactamase production is the most common resistance mechanism in staphylococci. The proportion of b+ subclinical CNS isolates (38%) was similar to those reported from subclinical mastitis or IMI in Finland (32%), Norway (36%) and Netherlands (37%) (Pitka¨la¨ et al., 2004; Østera˚ s et al., 2006; Sampimon, 2009).
  + Overall, resistance to other antimicrobials than penicillin was uncommon, and was markedly lower than in other studies for example for erythromycin, oxacillin and tetracycline
  + The prevalence of b-lactamase producing isolates varied markedly between CNS species, and was significantly higher in S. epidermidis and S. haemolyticus (40%), than in S. simulans and S. chromogenes where none or a few of the isolates produced b-lactamase
  + the distribution of CNS species differed between clinical and subclinical mastitis indicating inter-species variation of pathogenicity and epidemiology. Overall, the prevalence of antimicrobial resistance was low, but some variation between CNS species was observed
* ***Generally helpful info/stuff from other studies***
* ***speciation*** 
  + genotypic (tuf)
* ***type of resistance*** 
  + Phenotypic
* ***methodology***
  + microdilution
  + cloverleaf to check for b-lactamase production

***Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk***

**Frey 2013**

* ***Country***
  + Switzerland
* ***Species described***
  + 19 different CNS
* ***Clinical or subclinical***
  + Both and differentiates
* ***Num. isolates***
  + 408
* ***overall findings*** 
  + Resistance to oxacillin (47.0% of the isolates) indicator of mec gene-mediated methicillin resistance, fusidic acid (33.8%), 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%) was detected. Resistance to oxacillin was attributed to the mecA gene in 9.7% of the oxacillin-resistant isolates. The remaining oxacillin-resistant CNS did not contain the mecC gene or mecA1 promoter mutations. The mecA gene was detected in Staphylococcus fleurettii, Staphylococcus epidermidis, Staph. haemolyticus, and Staph. xylosus. Resistance to tetracycline was attributed to the presence of tet(K) and tet(L), penicillin resistance to blaZ, streptomycin resistance to str and ant(6)-Ia, and erythromycin resistance to erm(C), erm(B), and msr
  + Oxacillin resistance was attributed to the mecA gene present in 9.7% (n = 19) of the oxacillin-resistant isolates (n = 196). The mecA gene was detected in Staph. fleurettii (11/12), Staph. epidermidis (6/15), Staph. haemolyticus (1/37), and Staph. xylosus (1/155) isolates. The mecA or mecC gene was not detected in the other 177 oxacillin-resistant isolates (90.3%;
* ***Generally helpful info/stuff from other studies***
  + Resistance to other antimicrobials correlated with the presence of the associated resistance genes (Table 5): the β-lactamase gene blaZ; the tetracycline efflux genes tet(L) and tet(K); the streptomycin adenyltransferase and nucleotidyltransferase genes ant(6)-Ia and str; the chloramphenicol acetyltransferase genes catpC221 and catpC223; the gentamicin acetyltransferase gene aac(6’)-Ie; the kanamycin-neomycin phosphotransferase genes aph(2’)-Ia and aph(3c)-III; the macrolide and lincosamide 23S rRNA methylase genes erm(B) and erm(C); the macrolide efflux gene msr; the lincosamide nucleotidyltransferase gene lnu(A); and the trimethoprim-resistant dihydrofolate reductase genes dfr(A), dfr(D), dfr(G), and dfr(K). In a few strains, resistance to erythromycin, clindamycin, streptomycin, gentamicin, chloramphenicol, and trimethoprim could not be explained by the presence of any of the tested genes, suggesting new antimicrobial resistance mechanisms in CNS
* ***speciation*** 
  + MALDI
* ***type of resistance***
  + phenotypic
  + genotypic
* ***methodology***
  + broth microdilution
    - chloramphenicol, ciprofloxacin, clindamycin, dalfopristin-quinupristin, erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, oxacillin, penicillin, rifampicin, streptomycin, sulfamethoxazole, tetracycline, tiamulin, trimethoprim, and vancomycin
  + genotypic
    - mecA
    - mecC

***Species distribution and in vitro antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitic milk***

**Taponen 2016**

* ***Country***
  + Finland
* ***Species described***
  + S. simulans (25.0 %), S. epider-midis (25.0 %), S. chromogenes (15.4 %), S. haemolyticus
* ***Num. isolates***
  + 400
* ***Clinical or subclinical***
  + Both
* ***overall findings*** 
  + *Penicil-lin resistance was the most common type of antimicrobial resistance. Staphylococcus epidermidis was the most resist-ant among the four major species*
  + *The majority of S. epidermidis isolates were resistant to benzylpenicillin. On the contrary, only few S. simulans isolates were penicillin-resistant. Phenotypic oxacillin resistance was found in all four main species, and 34% of the isolates were oxacillin resistant. However, only 21 isolates (5%) were positive for the mecA gene. Of these, 20 were S. epidermidis and one S. sciuri. mecC positive isolates were not found.*
  + *Staphylococcus epidermidis differed from the three other major CoNS species as resistance to the tested antimicrobials was common, several isolates were multidrug resistant, and 19% of the isolates carried the mecA gene encoding methicillin resistance*
  + *The four major CoNS species differed in their in vitro antimicrobial susceptibility. Among them, antimicrobial resistance was most common in S. epidermidis*
* ***Generally helpful info/stuff from other studies*** 
  + The most common resist-ance among bovine CoNS is production of β-lactamase which confers resistance to benzylpenicillin and ami-nopenicillins, but also resistance towards aminoglyco-sides, tetracyclines, and macrolides has been reported
* ***Speciation***
  + Ribotyping and MALDI-TOF MS
* ***type of resistance***
  + phenotypic
* ***methodology***
  + broth microdilution
    - penicillin, cephalothin, oxacillin, eryth-romycin, chloramphenicol, clindamycin, tetracycline, gentamicin, neomycin, streptomycin, and trimethoprim/sulfamethoxazole. In addition, MICs for virginiamycin, vancomycin and avilamycin in dataset 1, and for fusidic acid, kanamycin, ciprofloxacin, trimethoprim, florfenicol, and cefoxitin in dataset 2

***Prevalence and antibiotic susceptibility of coagulase-negative Staphylococcus species from bovine subclinical mastitis in dairy herds in the central region of Argentina***

**Raspanti 2016**

* ***Country***
  + Argentina
* ***Species described***
  + Haemolyticus, chromogenes, xylosus and warneri to some extent
* ***Num. isolates***
  + 219
* ***Clinical or subclinical***
  + Doesn’t specify
* ***overall findings*** 
  + S. chromogenes and S. haemolyticus showed a very high proportion of isolates resistant to penicillin
  + One hundred and thirteen strains (51.6%) of the 219 strains were resistant to penicillin; The MIC90 value for this antibiotic was higher than 8g/ml, well above the recommended breakpoint. Thirty (13.7%) of all CNS isolates tested were resistant to oxacillin; the MIC90 value for this antibiotic was 0.5g/ml. Resistance to oxacillin was attributed to the presence of the mecA gene in 2 of 12 (16.7%) of the oxacillin-resistant isolates with a MIC >0.5g/ml. Moderate resistance to erythromycin and tetracycline was detected among CNS isolates, 29.2% and 30.1%,
* ***Generally helpful info/stuff from other studies***
* ***Speciation***
  + Genotypic (groEL gene)
* ***type of resistance***
* ***methodology***
  + broth microdilution

***Identification of variable traits among the methicillin resistant and sensitive coagulase negative staphylococci in milk samples from mastitic cows in India***

**Mahato 2017**

* ***Country***
  + India
* ***Species described***
  + 10 different CoNS speciesS. sciuri,S. haemolyticus, S. chromogenes,S. saprophyticus,S. xylosus,S. simulans,S. agnetis,S. epidermidis,S. gallinarum,andS. cohinii
* ***Num. isolates***
  + 62
* ***Clinical or subclinical***
  + Only clinical
* ***overall findings*** 
  + S. sciuriandS. haemolyticusappeared asthe most predominant species in which maximum isolates weremethicillin resistant. But doesn’t really do between-specie comparison
  + High resistance wasobserved against oxacillin and cefoxitin, whereas all isolates were susceptible towardvancomycin and linezolid
  + Fifty three isolates were methicillin resistant and 9 isolateswere sensitive as determined by oxacillin susceptibility assay. The methicillin resistancegene,mecAwas found in 95.16% isolates and staphylococcal cassette chromosomemec(SCCmec)
* ***Generally helpful info/stuff from other studies***
* ***speciation*** 
  + genotypic
* ***type of resistance*** 
  + genotypic and phenotypic
* ***methodology***
  + genotypic
    - mecA, mecC, vanA
  + phenotypic
    - the disk diffusion assay was donefor 8 antibiotics; clindamycin (2μg), erythromycin (15μg),gentamicin (10μg), ciprofloxacin (5μg), tetracycline (30μg),rifampicin (5μg), cefoxitin (30μg), and teicoplanin (30μg).All the antibiotic disks were procured from Himedia, Mumbai,India. The MICs against oxacillin (Sigma, St. Louis, MO, UnitedStates), vancomycin (Sigma, St. Louis, MO, United States) andlinezolid (Sigma, St. Louis, MO, United States) were evaluatedby micro-broth dilution method

***Prevalence and genetic basis of antimicrobial resistance in non-aureus staphylococci isolated from Canadian dairy herds***

**Nobrega 2018**

* ***Country***
  + Canada
* ***Species described***
  + 25 different species
* ***Num. isolates***
  + 1702 isolates/89 herds from 6 provinces (405 genotypic analysis)
* ***Clinical or subclinical***
  + Both, and differentiate (majority subclinical)
* ***overall findings*** 
  + Objectives were to determine: (1) phenotypic and genotypic prevalence of drug-specific resistance for 25 species of non-aureus staphylococci, and (2) associations between presence of resistance determinants and antimicrobial resistance. Broth micro-dilution was used to determine resistance profiles for 1,702 isolates from 89 dairy herds. Additionally, 405 isolates were sequenced to screen for resistance determinants. Antimicrobial resistance was clearly species-dependent. Resistance to quinupristin/dalfopristin was common in Staphylococcus gallinarum (prevalence of 98%), whereas S. cohnii and S. arlettae were frequently resistant to erythromycin (prevalence of 63 and 100%, respectively). Prevalence of resistance was 10% against β-lactams and tetracyclines. In contrast, resistance to antimicrobials critically important for human medicine, namely vancomycin, fluoroquinolones, linezolid and daptomycin, was uncommon (<1%)
  + The objectives of this study were to: (1) estimate prevalence of drug-specific AMR in NAS isolated from Canadian dairy cows, (2) characterize genetic determinants of AMR associated with various drugspecific resistance profiles, and (3) study the association between the presence of resistance determinants and AMR in NAS isolated from Canadian dairy herds
  + The prevalence of AMR was highest for tetracycline, penicillin (10% for each), and erythromycin (6%) (Table 1). Staphylococcus arlettae had the highest prevalence of AMR, particularly against penicillin (61%), ampicillin (23%), erythromycin (100%), pirlimycin (18%) and clindamycin (99.9%)
  + prevalence of AMR in NAS isolated from Canadian dairy herds was higher for tetracycline, penicillin and erythromycin compared to other antimicrobials, and was NAS species-dependent. S. arlettae had the highest prevalence of MDR. No isolate was resistant to vancomycin, linezolid or fluoroquinolones. The most frequently identified resistance determinants were mutations in the folP gene and MDR efflux pumps, which were present in all isolates and not associated with the MDR phenotype. The blaZ, mecA, fexA, erm, mphC, msrA, and tet genes were associated with drug-specific AMR. There were specific residues in gyrB for NAS species intrinsically resistant to novobiocin
  + Resistance to vancomycin, fluoroquinolones, linezolid and daptomycin was absent or uncommon. These drugs are considered critically important in human medicine and their use is restricted in foodproducing animals in several countries. Resistance against highly important antimicrobials, frequently used in dairy herds (e.g., penicillins and tetracyclines) was relatively common. Some NAS species had species-specific patterns of resistance against specific antimicrobials. Moreover, prevalence of AMR genetic determinants was also species-specific; for example, the prevalence of the mecA elements was estimated to be 17% in S. epidermidis, but close to zero for other species isolated from bovine milk
  + Genetic basis: The most common genetic basis of resistance included the presence of AMR-associated residues in the dihydropteroate synthase gene deduced amino acid sequence (folP gene; all sequenced isolates, ranging from two to six residues), the putative multidrug export ATP-binding/permease protein SAV1866 (99% of the isolates), the major facilitator superfamily (MFS) multidrug efflux transporter NorA represented by the norA gene (91% of the isolates) and the DHA sub-family of MFS transporters (61% of the isolates). Drug-specific efflux pumps-coding genes identified included tet38, tetK, and tetL (21, 12, and 3% of all NAS sequenced, respectively), the mrsA gene (42 isolates; 10%), and the chloramphenicol/florfenicol efflux MFS transporter FexA represented by the fexA gene (five isolates; 1%). Nonsynonymous mutations in the quinolone resistance-determining region (QRDR) previously reported as associated with AMR were present for the parC and parE genes in isolates of S. devriesei and S. epidermidis, respectively. gyrB residues associated with resistance against aminocoumarins were present as the dominant pattern for several species intrinsically resistant to novobiocin. No AMR-associated residue was detected in the deduced amino acid sequence of the rpoB, rpoC and gyrA genes. erm genes, which encode for rRNA adenine N-6- methyltransferases, were present exclusively in S. epidermidis, S. cohnii, S. equorum, and S. chromogenes. No MLS-resistance mechanisms were present in S. gallinarum. The ABC-transporter encoding vgaA was detected in six isolates, whereas the virginiamycin B lyase encoding vgbB was present in a single S. xylosus isolate. van elements associated with vancomycinresistance were not detected. The mecA1 gene was present in all S. sciuri isolates and the mecA sf was present in all S. fleuretti. No mutation was detected in the promoter region of these genes
* ***Generally helpful info/stuff from other studies*** 
  + Results for NAS (or CNS) were historically reported as a group. However, it recently became clear that these species should be treated individually, as risk factors can be species-specific (De Visscher et al., 2017). In our study, prevalence of AMR was clearly species-dependent.
  + It is noteworthy that the vast majority of studies designed to estimate the prevalence of AMR in NAS isolated from dairy herds are regional and limit screening of resistance determinants to resistant isolates, usually by PCR or a similar approach. This methodology has two major limitations. First, PCR and its variants are usually designed to target few AMR genes (ARGs), limiting results to screened elements. Second, estimated prevalences of ARGs refer to the presence of genetic determinants in the phenotypically resistant population, whereas the same in the phenotypically susceptible population is unknown. The availability of such information would be important for interpreting patterns and trends of AMR, provide an estimate of the impact of various genes in drug-specific resistance, serve as a basis of risk assessment, and determine effects of interventions for controlling AMR
  + Irrespective of the apparent zoonotic potential that NAS isolates obtained from mastitic milk may have (Thorberg et al., 2006), NAS of animal origin are believed to be important reservoirs of ARGs, which is of utmost importance for human and veterinary medicine. Indeed, the mecA gene was frequently detected in S. epidermidis, the most common staphylococcal species recovered from humans (Becker et al., 2014).
* ***Speciation***
  + rpoB/genotypic
* ***type of resistance***
  + genotypic and phenotypic
* ***Methodology***
  + Phenotypic
    - microdilution
    - Antimicrobials and concentrations evaluated were ampicillin (0.12–8µg/ml), chloramphenicol (2–16µg/ml), ceftiofur (0.5–4µg/ml), cephalothin (2–16µg/ml), ciprofloxacin (1–2µg/ml), clindamycin (0.5–2µg/ml), daptomycin (0.5–4µg/ml), erythromycin (0.25–4µg/ml), gentamicin (2– 16µg/ml), levofloxacin (0.25–4µg/ml), linezolid (1–8µg/ml), moxifloxacin (0.25–4µg/ml), nitrofurantoin (32–64µg/ml), oxacillin + 2% NaCl (0.25–4µg/ml), penicillin (0.06– 8µg/ml), penicillin/novobiocin (1/2–8/16µg/ml), pirlimycin (0.5–4µg/ml), quinupristin/dalfopristin (0.5–4µg/ml), rifampin (0.5–4µg/ml), tetracycline (2–16µg/ml), tigecycline (0.03–0.5µg/ml), trimethoprim/sulfamethoxazole (0.05/9.5– 4/76µg/ml), and vancomycin (0.25–32µg/ml
  + Genotypic
    - WGS, whole list of stuff they were looking for

***Antimicrobial resistance and virulence characteristics in 3 collections of staphylococci from bovine milk samples***

**Fergestad 2021**

* ***Country***
  + Belgium and Norway
* ***Species described***
  + 16 different NAS species, aureus
* ***Num. isolates***
  + 272 including aureus and NAS
* ***Clinical or subclinical***
  + both
* ***overall findings*** 
  + Antimicrobial resistance was com-mon in Staphylococcus epidermidis and Staphylococcus haemolyticus from all different groups, with resistance to trimethoprim-sulfonamide frequently occurring in Staph. epidermidis and Staph. haemolyticus as well as in Staph. aureus. Resistance to penicillin was most frequently observed in group of isolates from Norway
  + Descriptive analyses of antimicrobial resistance char-acteristics in all 3 sample groups showed that these were more widespread in several NAS species compared with Staph. aureus, apart from the MRSA isolates.
  + Antimicrobial resis-tance was frequently observed in Staph. epidermidisand Staph. haemolyticus regardless of sample group
* ***Generally helpful info/stuff from other studies***
* ***Speciation***
  + MALDI
* ***type of resistance***
  + phenotypic and genotypic
* ***Methodology***
  + Phenotypic
    - Disc diffusion
    - ampicillin (10 μg), amoxicillin and clavulanic acid (20 + 10 μg), ciprofloxacin (5 μg), clindamycin (10 μg), erythromycin (15 μg), gentamicin (10 μg), linezolide (10 μg), penicillin (1 U), trimethoprim (5 μg), sulfonamide and trimethoprim (19:1, 25 μg), and tetracycline (30 μg). Cefoxitin (30 μg) was used for determination of phenotypic methicillin resistance
  + Genotypic
    - mecA, mecC PCR

***Antimicrobial susceptibility of staphylococci from bovine milk samples in routine microbiological mastitis analysis in Finland***

**Taponen 2023**

* ***Country***
  + Finland
* ***Species described***
  + aureus and NAS
* ***Num. isolates***
  + 260 aureus, 244 NAS
* ***Clinical or subclinical***
  + Probably both, not known/specified
* ***overall findings*** 
  + 26.6% of the isolates (18.5% of S. aureus and 35.2% of all NAS) carried the blaZ gene. Penicillin resistance, based on disk diffusion, was lower: 18.8% of all the isolates (9.3% of S. aureus and 28.9% of all NAS) were resistant. Based on the nitrocefin test, 21.5% of the isolates produced beta-lactamase (11.6% of S. aureus and 32.0% of all NAS). Between the Staphylococcusspecies, the proportion of penicillin-resistant isolates varied, being lowest in S. simulans and highest in S. epidermidis. Resistance to antimicrobials other than penicillin was rare. Of the eight NAS isolates carrying the mecA gene, six were S. epidermidis
  + our results support earlier findings that penicillin resistance is the only significant form of antimicrobial resistance among mastitis-causing staphylococci in Finland
* ***Generally helpful info/stuff from other studies*** 
  + In Finland, prudent antimicrobial use guidelines for mastitis therapy are followed: Bacteriologic analysis of milk samples before initiation of antimicrobial treatment is a common practice and penicillin is the drug of choice in mastitis caused by Gram-positive bacteria (7). Additionally, selective dry cow therapy (SDCT) has always been implemented, with only approximately one-fourth of cows receiving antibiotic dry cow treatment at the end of lactation
  + that a direct comparison of the results between studies is difficult, as study populations, sample collection, and sources of the isolates and methods for antimicrobial susceptibility testing differ. Another explanatory factor for differences in penicillin resistance figures may be related to the distribution of NAS species
  + Huge differences in penicillin resistance between the Staphylococcus species were detectable both in ours and other studies
  + In our study, the lowest proportion of penicillin-resistant isolates was in S. simulans, and the highest in S. epidermidis, based on disk diffusion, nitrocefin production, and blaZ carriage. Many other studies have also shown S. simulans to be mainly susceptible to penicillin and shown S. epidermidis to be the staphylococcal species most resistant to penicillin and to several other antimicrobials (12,36,37). Multidrug resistance has also been most common in S. epidermidis(11,40). In addition, S. epidermidis is the Staphylococcus species that most commonly carries the mecA gene coding for methicillin resistance (11,36,37), and consistently, of the eight mecA-positive isolates in the current study, six were S. epidermidis
* ***Speciation***
  + MALDI
* ***type of resistance***
* ***Methodology***
  + Phenotypic susceptibility against cefoxitin, ceftiofur, enrofloxacin, gentamycin, oxacillin, penicillin, and tetracycline was evaluated by disk diffusion method
  + presence of blaZ, mecA, and mecC genes investigated by PCR

***Antimicrobial resistance and virulence profiles of staphylococci isolates from clinical bovine mastitis***

**Yang 2023**

* ***Country***
  + China
* ***Species described***
  + 15 species CNS and aureus
* ***Num. isolates***
  + 172 S. aureus and 160 CNS isolates
* ***Clinical or subclinical***
  + clinical
* ***overall findings*** 
  + The S. aureus and CNS isolates showed high resistance against penicillin, followed by erythromycin and tetracycline.
  + Resistance to penicillin was attributed to the presence of blaZ, erythromycin resistance to ermC (alone or combined with ermB) and tetracycline resistance to tetK (alone or combined with tetM)
  + The S. aureusisolates showed highest resistance rate to penicillin (101, 58.7%), followed by erythromycin (38, 22.1%), tetracycline (26, 15.1%), gentamicin (18, 10.5%), ciprofloxacin (15, 8.7%), and chloramphenicol (10, 5.8%)
  + CNS isolates displayed high resistance to penicillin (114, 71.3%), followed by erythromycin (46, 28.8%), tetracycline (31, 19.4%), gentamicin (15, 9.4%), chloramphenicol (9, 7.9%), ciprofloxacin (4, 2.5%), and cefoxitin (2, 1.3%
  + In S. aureus isolates, the blaZ was detected in 105 (61.0%) isolates. All penicillin-resistant S. aureus isolates carried blaZ. Besides, 4 penicillin-susceptible isolates also contained this gene. The tetK and tetM were determined in 21 (12.2%) and 17 (9.9%) isolates, respectively. All tetKpositive (alone or combined with tetM) isolates showed resistance to tetracycline. Five tetracycline-resistant S. aureus isolates were negative for tetK or tetM. Additionally, genes ermC and ermB were found in 38 (22.1%) and 23 (13.4%) S. aureus isolates, respectively. And all erythromycin-resistant isolates harbored ermC alone or in combination with ermB. None of the isolates were positive for the mecA, mecC or ermA
  + Among the 160 CNS isolates evaluated, the blaZ was found in 111 (69.4%) isolates and all of them showed resistance to penicillin. Two S. equorum and 1 S. simulans that were resistant against penicillin were negative for blaZ. Importantly, both of the methicillin-resistant isolates, 1 S. equorum and 1 S. saprophyticus, carried mecA. The tetKand tetM were determined in 28 (17.5%) and 20 (12.5%) CNS isolates, respectively. All tetK-carrying (alone or combined with tetM) isolates showed resistance to tetracycline. Three tetracycline-resistant isolates, including 1 S. chromogenes, 1 S. haemolyticus and 1 S. saprophyticus, did not harbored tetK or tetM. Moreover, ermC and ermB were detected in 45 (28.1%) and 27 (16.9%) CNS isolates, respectively. All ermC-carrying (alone or combined with ermB) isolates displayed resistance to erythromycin. One erythromycin-resistant S. equorum was negative for ermC or ermB. None of the CNS isolates were positive for the mecC or ermA
  + The S. aureus and CNS isolates displayed high frequencies of phenotypic and genotypic resistance to penicillin, erythromycin and tetracycline
* ***Generally helpful info/stuff from other studies*** 
  + Antimicrobial resistance of staphylococci are mainly attributed to various resistant determinants, such as genes blaZ and mecA for β-lactams resistance, tets for tetracyclines resistance, and erms for macrolides resistance.
* ***Speciation***
  + Genotypic/16S
* ***type of resistance***
  + phenotypic and genotypic
* ***Methodology***
  + Disc diffusion and E-test
    - The panel of antimicrobial agents (Oxoid) included penicillin (10 U), cefoxitin (30 μg), gentamicin (10 μg), erythromycin (15 μg), tetracycline (30 μg), ciprofloxacin (5 μg), nitrofurantoin (300 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), quinupristin/dalfopristin (15 μg), and linezolid (30 μg). Susceptibility to cefoxitin was used to detect the methicillin-resistance phenotype. The E-test strips (Liofilchem, Roseto, Italy) were used to detect the vancomycin (0.016 to 256 μg/mL) susceptibility of the staphylococcal isolates
  + Genotypic
    - The resistance genes for penicillin (blaZ), methicillin (mecAand mecC), tetracycline (tetK and tetM), and erythromycin (ermA, ermB, and ermC) were tested by PCR

***Antimicrobial susceptibility of coagulase-negative Staphylococcus species isolated from bovine milk***

**Sawant 2009**

* ***Country***
  + US
* ***Species described***
  + 10 different NAS
* ***Num. isolates***
  + 168
* ***Clinical or subclinical***
  + Doesn’t specify
* ***overall findings*** 
  + S. epidermidis was the only species that exhibited low susceptibility to certain antimicrobials
  + A total of 17 of 37 (46%) S. epidermidis, 11 of 61 (18%) S. chromogenes and one of 37 (3%) S. hyicus exhibited phenotypic resistance to ampicillin (0.5 mg/ml). All these isolates produced b-lactamase, all carried blaZ gene
  + Of 37 S. epidermidis evaluated, 13 (35%) exhibited efflux-based resistance to erythromycin (16 mg/ml) encoded by msrA and one isolate carried ermC encoding ribosomal methylase-based resistance to both erythromycin (64 mg/ml) and pirlimycin (64 mg/ml); epi had blaZ, msrA, mecA, ermC
  + Studying CNS at the species level can provide valuable information about species-specific differences that can be vital data for effective mastitis therapy and management
  + The present study revealed differences in antimicrobial susceptibility among the CNS species evaluated. The majority of CNS except S. epidermidis were susceptible to commonly used antimicrobials for mastitis treatment and prevention. Resistance to ampicillin, erythromycin, methicillin and pirlimycin was observed in S. epidermidis. Only constitutive b-lactamase producing CNS was readily identified on the MIC panel.
* ***Generally helpful info/stuff from other studies***
  + The presence of resistance to both erythromycin (macrolide) and pirlimycin (lincosamide) in one strain of S. epidermidis (Table 2) is significant since this resistance was mediated by ribosomal methylase (ermC based, GenBank Accession no. EF015602). This resistance determinant is plasmid-based and observed among different CNS suggesting horizontal transfer (Luthje and Schwarz, 2006). Resistance to pirlimycin has clinical relevance as it is a very efficient and widely used antimicrobial for mastitis therapy in dairy cows
* ***speciation*** 
  + API Staph
* ***type of resistance*** 
  + both phenotypic and genotypic
* ***methodology***
  + microdilution
    - Antimicrobials used in this study were ampicillin (0.06–64 mg/ml), ceftiofur (0.06–64 mg/ml), cephalothin (0.06–64 mg/ml), erythromycin (0.06–64 mg/ml), oxacillin (0.06–64 mg/ml), and pirlimycin (0.06–64 mg/ml).
  + Selective media to check for b-lactamase production
  + Genotypic
    - ermA; ermB, ermC, msrA,
    - any producting b-lactamase: blaZ, mecA
    - epidermidis: icaA, icaB

***Genomic characterization of coagulase-negative staphylococci including methicillin-resistant Staphylococcus sciuri causing bovine mastitis***

**Khazandi 2018**

* ***Country***
  + Australia
* ***Species described***
  + S. chromogenes (n=1) S. fleurettii (n=1), S. haemolyticus (n=2), S. sciuri (n=5), S. simulans (n=1) S. succinus (n=2) and S. xylosus (n=2). Five of the isolates (S. fleuretti, S. haemolyticus S. sciuri and two S. succinus)
* ***Num. isolates***
  + **Case report, really**
  + **14 ID’d as MR, all from 1 farm**
* ***Clinical or subclinical***
  + Clinical
* ***overall findings*** 
  + Methicillin-resistant coagulase-negative staphylococci (MRCoNS) have recently emerged as a significant cause of bovine mastitis worldwide. Fourteen CoNS isolates from cases of bovine mastitis from a single dairy farm in Australia were identified as MRCoNS. Five of the isolates (S. fleuretti, S. haemolyticus S. sciuri and two S. succinus) were mecA-positive. Four of S. sciuri isolates carried mecA homolog. These were similar ot isolates humans, a squirrel and a cereal crop (rice. In conclusion, CoNS, in particular S. sciuri, may act as a reservoir for SCCmec elements that can easily be spread between different host species by direct cross-infection
  + CoNS, in particular S. sciuri, may act as a reservoir for SCCmec elements that can easily be spread between different host species by direct cross-infection.
  + A high degree of genome homogeneity was demonstrated between mammalian and plant isolates of methicillin-resistant S. sciuri suggesting low host specificity. Our findings indicate S. sciuri and other MRCoNS could act as a resevoir for gene cassettes containing mecA or mecA homologues in dairy cattle, as well as additional antimicrobial resistance genes with potential for bi-directional transmission between humans and cows
* ***Generally helpful info/stuff from other studies***
* ***Speciation***
  + 16s, MALDI
* ***type of resistance***
  + phenotypic and genotypic
* ***Methodology***
  + Microdilution
  + Genotypic
    - WGS
    - mecA by PCR

***Characterization of coagulase-negative staphylococcus species from cows’ milk and environment based on bap, icaA, and mecA genes and phenotypic susceptibility to antimicrobials and teat dips***

**Piessens 2012**

* ***Country***
  + Belgium
* ***Species described***
  + 22 different NASM; **majority from environmental source**
* ***Num. isolates***
  + 82 total, 33 milk
* ***Clinical or subclinical***
  + Doesn’t specify
* ***overall findings*** 
  + compared between typically contagious CNS species (Staphylococcus chromogenes, Staphylo-coccus epidermidis, Staphylococcus haemolyticus, and Staphylococcus simulans) and those rarely causing IMI (Staphylococcus sciuri, Staphylococcus equorum, and others) to find possible associations with pathogenicity
  + Antimicro-bial resistance was mainly against erythromycin (23%) or oxacillin (16%), and was detected more often in the environmental species
  + mecA genes were detected significantly more in isolates from CNS species typically living in the cows’ environment than in isolates from IMI-causing species
  + Environmental CNS were significantly more mecA-positive (24.0%) than the IMI-causing species (3.6%; P < 0.001). The mecA was detected in 33.3% (8/24) of Staph. epidermidis isolates, and was common in Staph. sciuri and Staphylococcus fleurettii
* ***Generally helpful info/stuff from other studies***
* ***speciation*** 
  + genotypic
* ***type of resistance***
  + phenotypic
  + genotypic
    - mecA
* ***methodology***
  + E-test, an inhibitory agar; ephalothin, enrofloxacin, erythromycin, gentamicin, and oxacillin, representing the cephalosporins, fluoroquinolones, macrolides, ami-noglycosides, and penicillins, respectively

* ***Country***
* ***Species described***
* ***Num. isolates***
* ***Clinical or subclinical***
* ***overall findings***
* ***Generally helpful info/stuff from other studies***
* ***Speciation***
* ***type of resistance***
* ***Methodology***

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) --- consistency with org vs. conventional in SAME study (when question is, is one population of isolates more resistant than another) – but maybe problematic to compare AMR BETWEEN studies

internal vailidity (comparision within org and conv farms in same study) good enough, but limited generalizability between studies using different sampling schemes, methodology

***AB susceptibility testing***

***Taponen 2023*** –

Compared different methods of testing for b-lactamase resistance; overall agreement between phenotypic and genotypic resistance tests was moderate to substantial, but they did find some discrepancies between methods of identifying penicillin resistance between phenotypic susceptibility by disk diffusion method, the nitrocefin test to assess beta-lactamase production, and PCR to detect the and the presence of *blaZ, mecA*, and *mecC* genes.

Agreement beyond chance, assessed by kappa coefficient, between phenotypic and genotypic resistance tests, was moderate to substantial. Some phenotypically penicillin-susceptible staphylococci carried the blaZ gene but isolates without blaZ or mecgenes rarely exhibited resistance, suggesting that the more reliable treatment choice may depend upon genotypic AMR testing

Agreement beyond chance, assessed by kappa coefficient, between phenotypic and genotypic resistance tests, was moderate to substantial. Some phenotypically penicillin-susceptible staphylococci carried the blaZ gene but isolates without blaZ or mecgenes rarely exhibited resistance, suggesting that the more reliable treatment choice may depend upon genotypic AMR testing

problems with just screening to see if blaZ is present – mec also confers penicillin resisteance, and is not looked for as often.

The occurrence of the mec genes is not routinely tested for in mastitis cases. Some blaZ-negative isolates may be mec positive and thus penicillin resistant. The only mecC-positive S. aureus isolate in our study was blaZ negative and was incorrectly classified as penicillin susceptible. Penicillin treatment of these cases would have led to treatment failure. The oxacillin disk-diffusion test better indicated mecA carriage than did the cefoxitin disk-diffusion test. However, many of the oxacillin disk-diffusion test results were false positive, i.e., mec-negative isolates showing resistance to oxacillin. Neither of the tests was, however, perfect for detecting mec-gene carriage. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and the Laboratory Standards Institute (CLSI) recommend cefoxitin disk for screening of mecA-mediated beta-lactam resistance in Staphylococcus aureus and NAS (25,45), but in our study it did not detect potential mecA-positive isolates very effectively. The cefoxitin disk is reported to perform better for mecC screening than does the oxacillin disk (46,47). The only mecC-positive S. aureus isolate in our study was detectable by both methods. We did not test for the recently detected mec-gene variant mecB (48), and other resistance mechanisms may also exist, such as mutations in the gdpP gene

**The discrepancy between blaZ-gene carriage and the phenotypic penicillin resistance detected in our and other studies in veterinary (9,50) and human (51,52) medicine is an interesting phenomenon. BlaZ-negative but phenotypically penicillin-resistant S. aureus isolates are scarce (9), but blaZ-positive isolates that are phenotypically penicillin sensitive commonly exist (9,50,51). Different tests typically show different sensitivities for detection of beta-lactamase production. Pitkälä et al. (53) compared six tests, using the blaZ PCR as the reference method. At least one method was always positive, supporting the potential for beta-lactamase production of the blaZ-positive isolates. Some authors report that tests based on detection of beta-lactamase production (e.g., nitrocefin test, clover leaf test) correlate better with the occurrence of the blaZgene than does the agar dilution method (54). Others have reported lower sensitivities for these tests (51,55). The results of the agar dilution and MIC methods depend on the set cut-off values. In our study, the agreement beyond chance between phenotypic penicillin resistance based on the disk diffusion test and occurrence of the blaZwas moderate to substantial, depending on the Staphylococcusspecies. One possible reason for phenotypically penicillin susceptible but blaZ-positive isolates is impaired function of the blaZ or its regulators, the blaI and blaR genes, because of sequence mutations (56).**

***Comparison between broth microdilution and agar disk diffusion methods for antimicrobial susceptibility testing of bovine mastitis pathogens***

Palladini 2023

A study comparing agreement between the broth microdilution (Sensititre Custom Plates) and the agar disk diffusion method for determining antimicrobial susceptibility of isolates from bovine mastitis found fair agreement overall (80.7%) between the two methods, but this varied based on the particular bacterial-antimicrobial combinations tested. No NAS species were tested, but there was satisfactory agreement (89 to 100%) for *S. aureus* and all antimicrobial agents tested.

The overall agreement between the two methods was found to be 80.7% with a Cohen's kappa coefficient of 0.397, thus indicating a fair concordance. BMD method and ADD method demonstrated a satisfactory agreement (89 to 100%) for S. aureus and S. marcescens and all antimicrobial agents tested. Low agreement was observed for S. uberis and rifampicin (20%), enrofloxacin (49%), penicillin (51%) and pirlimycin (52%), E. coli and  (20%), S. dysgalactiae and enrofloxacin (44%), S. agalactiae and rifampicin (25%).

The ADD has long been used in veterinary diagnostics due to its easy use, low cost and flexibility in the type and number of antimicrobial agents to be tested. However, some limitations affect the overall performance of this method: it allows to test a single value of antimicrobial concentration, it depends on the operator for the execution, reading and interpretation of the diameter of inhibition zone, and it only provides a qualitative result (susceptible, intermediate or resistant) (Klement et al., 2005). On the contrary, the broth microdilution method allows to obtain a quantitative result, namely the Minimum Inhibitory Concentration (MIC), and has the advantage of being suitable for use in automated systems (Lazou and Chaintoutis, 2023). One of the systems available on the market for the evaluation of bacterial antimicrobial susceptibility is the Sensititre System (Thermo Fisher TREK Diagnostic Systems, Inc., Cleveland, OH, USA). This system provides “ready-to-use” microplates containing specific antimicrobials molecules generally used for bovine mastitis therapy. Moreover, the system is associated with an optical density reader which allows to standardize the inoculum, an automatic dispenser and an instrument for reading the results, reducing the possibility of human errors.

various bacterial-antimicrobial combinations,

***Diagnostic accuracy assessment of Sensititre and agar disk diffusion for determining antimicrobial resistance profiles of bovine clinical mastitis pathogens***

**Saini 2011**

In a study comparing Sensititre (broth microdilution) and disk diffusion for determining antimicrobial resistance profiles of clinical mastitis pathogens, agreement was good between the two methods for most isolate-antimicrobial MIC combinations. An important caveat in this study was that the diagnostic accuracy was low for *S. aureus*-ceftiofur/oxacillin combinations with either testing method.

Essential agreement between Sensititre automatic MIC readings and MIC readings obtained by the broth microdilution test method was 87%. Essential agreement between Sensititre automatic and manual MIC reading methods was 97%. Furthermore, the ADD test method and Sensititre MIC method exhibited 92 and 91% categorical agreement (sensitive, intermediate, resistant) of results, respectively, compared with the reference method. However, both methods demonstrated lower agreement for E. coli-ampicillin/cephalothin combinations than for Gram-positive isolates. In conclusion, the Sensititre and ADD methods had moderate to high diagnostic accuracy and very good essential and categorical agreement for most udder pathogen-antimicrobial combinations and can be readily employed in veterinary diagnostic laboratories.

***Assessment of Accuracy of Disk Diffusion Tests for the Determination of Antimicrobial Susceptibility of Common Bovine Mastitis Pathogens: A Novel Approach***

**Klement 2005**

A study comparing dilution methods to determine MIC and disk diffusion diameters for mastitis bacterial isolates found low correlation between the two methods for *S. aureus* and erythromycin, and *S. aureus* and neomycin.

S. aureus and erythromycin and neomycin

A novel approach was used to assess disk diffusion accuracy for determination of antibiotic susceptibility of various bovine mastitis pathogens (Escherichia coli, Staphylococcus aureus, Staphylococcus chromogenes, and Streptococcus dysgalactiae). MIC and disk diffusion diameters were compared for 587 bovine mastitis bacterial isolates collected in Israel and 3,186 drug–organism combinations. Results were analyzed by ROC curves, Bayesian statistics, and standard descriptive methods. Low correlation was observed between results of disk diffusion and MIC for S. dysgalactiae and all antimicrobial agents, S. aureus and erythromycin and neomycin, and E. coli and gentamicin, neomycin, and polymyxin B

1. Why we care about AMR?
   1. 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).
   2. Want to make sure antimicrobials still work to relieve suffering of animals, cure infections and keep being able to provide large volume of high quality fluid milk
2. Why we care about AMR in particular in Staph/want to monitor
   1. Virulence genes found to cause disease in people and animals have been demonstrated specifically in NAS isolates from bovine IMI
      1. Unal 2012
         1. Staphylococcal enterotoxin, MRSA, Panton-Valentine leukocidin genes from cows and sheep with SCM
      2. *Park 2011:* investigated the presence of 19 classical and newly described staphylococcal superantigen (SAg) genes in CNS isolates from bovine intramammary infections (IMI). A total of 263 CNS representing 11 different Staphylococcus spp. were examined, and 31.2% (n = 82) of CNS isolates had one or more SAg genes; there were 21 different SAg gene combinations. The most prevalent combination of SAg genes (seb, seln and selq; n = 45) was found in S. chromogenes, S. xylosus, S. haemolyticus, S. sciuri subsp. carnaticus, S. simulans and S. succinus. The genes for SAgs appear to be widely distributed amongst CNS isolated from bovine IMI.
   2. It has been recently reported that CoNS harbor staphylococcal cassette chromosome mec (SCCmec) elements which act as a reservoirs of antimicrobial resistance determinants and which can be transferred via direct transmission of resistant pathogens between different hosts and/or lateral transfer of resistance genes through genetic recombination
      1. Li et al. 2015; Khazandi 2018
   3. CoNS tend to be more resistant to antibacterial agents than S. aureus and can easily develop multi-resistance, in study below
      1. Taponen 2009: Coagulase-negative staphylococci as cause of bovine mastitis—Not so different from Staphylococcus aureus?.
   4. Previous studies also demonstrated that plasmids carrying various antimicrobial resistance genes such as cfr, erm(C), erm(T), lnu(A), or dfrK were identified in both MRSA and CoNS, indicating horizontal transmission of the plasmids across bacterial species in various environments
      1. Shen 2013: Presence and dissemination of the multiresistance gene cfr in Gram-positive and Gram-negative bacteria
      2. Feßler, 2018: Mobile macrolide resistance genes in staphylococci
      3. Feßler, 2018: Small antimicrobial resistance plasmids in livestock-associated methicillin-resistant Staphylococcus aureus CC398.
   5. Furthermore, Cuny et al. reported occurrence of cfr-carrying plasmids in CoNS isolates from calves and veterinarians along with the transferability of the plasmids among different staphylococcal species
      1. Cuny, 2017: Occurrence of cfr-mediated multiresistance in staphylococci from veal calves and pigs, from humans at the corresponding farms, and from veterinarians and their family members
3. AM generally selects for resistance
   1. Parker 2024:
      1. The causal pathway from bacterial susceptibility to resistance is not simple, and dissemination is cyclical rather than linear. Amplification of AMR occurs in healthcare environments and on farms where frequent exposure to antimicrobials selects for resistant bacterial populations
   2. From pam’s 2007: It was suggested that the usage of antimicrobial drugs in food animals might affect human health by increasing the risk of antimicrobial residues or by influencing the generation or selection of drug-resistant foodborne pathogens (Yan and Gilbert, 2004)
   3. Mathew 2007: The use of antimicrobial compounds in food animal production provides demonstrated benefits, including **improved animal health**, higher production and, in some cases, reduction in foodborne pathogens. However, use of antibiotics for agricultural purposes, particularly for growth enhancement, has come under much scrutiny, as it has been shown to contribute to the increased prevalence of antibiotic-resistant bacteria of human significance. **The transfer of antibiotic resistance genes and selection for resistant bacteria can occur through a variety of mechanisms, which may not always be linked to specific antibiotic use. Prevalence data may provide some perspective on occurrence and changes in resistance over time; however, the reasons are diverse and complex.**
   4. *Antimicrobial Use and Antimicrobial Resistance: A Population Perspective*
      1. Marc Lipsitch and Matthew H. Samore
      2. the well-supported idea that the use of antimicrobial agents is a powerful selective force that promotes the emergence of resistant strains …. cumulative effect of using these antibiotics has clearly been to increase the prevalence of resistance in the population as a whole.
4. AM use on dairy farms, especially mastitis
   1. Mastitis is one of the most frequent infectious diseases in dairy cattle and is the primary cause of antimicrobial drug usage in adult dairy cows (Pol and Ruegg, 2007 AB usage one)
   2. 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), and lincosamides (pirlimycin) (FDA–Center for Veterinary Medicine, 2004)
5. Organic vs. conventional farms provide an interesting arena in which to explore pressure of AM use on AMR prevalence

* **Intro material (set up the premise)**
  + 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)

*From Call 2008*

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. In some cases, such as fluoroquinolones, antimicrobials can select for de novo chromosomal mutations that confer resistance and allow for relatively rapid emergence of resistant strains

One way to assess the effect of antimicrobial use on AMR is to contrast systems that employ different production strategies. For example, ‘organic’ dairies employ little to no antimicrobials compared with ‘conventional’ production environments. For the US dairy industry, conventional dairies use antimicrobials more frequently in all age categories of dairy cattle compared to organic dairies (Zwald et al., 2004; Pol and Ruegg, 2007b), and land use requirements for organic certification call for at least 3 years of antimicrobial-free operation

*From Pol and Ruegg*

Mastitis is one of the most frequent infectious diseases in dairy cattle and is the primary cause of antimicrobial drug usage in adult dairy cows (Pol and Ruegg, 2007).

The goal of this study was to analyze relationships between antimicrobial usage at the farm level and antimicrobial susceptibility of mastitis pathogens

* **Literature showing/suggesting AB usage = selection pressure**
  + - (Lopez-Lozano et al., 2000)
    - *“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.