***Current state of knowledge***

* ***NAS are important and widespread mastitis pathogen***
  + **NAS are widespread**
    - ***“It is well established that NAS is the most commonly isolated group of bacteria from bovine milk samples worldwide; therefore, understanding their effect on udder health is essential”***
    - De Visscher 2017: 299/300 BTM samples in Belgium
    - Katholm 2012: present on 100% of all 4,258 Danish dairy herds (BTM)
    - *Citations showing most common SCM pathogen, from Valckenier 2021:*
      * *In the past few decades, non-aureus staphylococci (NAS) have become the most common pathogen isolated from subclinical mastitis cases in dairy cows in many regions (Pitkälä et al., 2004; Piepers et al., 2007; Pyörälä and Taponen, 2009; Reyher et al., 2011)*
    - *Non-aureus staphylococci are the most frequently isolated bacteria causing IMI in dairy cows worldwide (from Wutyack, JDS)*
      * Sampimon et al., 2009b; Schukken et al., 2009; Condas et al., 2017 (26% quarter level prevalence as a group in large, trans-Canadian study).
    - Valckenier 2020
      * 76.4% of the IMI in early-lactating heifers (89 IMI); found in 21% of all quarters (infected and non-infected combined) in Belgium for first sampling day
      * 53.1% of the culture-positive samples in early-lactating heifers, found in 10.5% of *all* quarters (infected and non-infected combined) in Belgium for second sampling day
    - Wuytack 2020 (November JDS)
      * 33% of ALL quartermilk samples were positive for NAS IMI (range: 18-50% per herd)
  + **NAS IMI are a problem… maybe**
    - **They are primarily associated with subclinical vs. clinical mastitis**
      * Persson Waller 2011, Heikkila 2018 *(got citations from Valckenier 2020)*
    - **But can cause mild clinical mastitis as well**
      * Taponen et al., 2006; Simojoki et al., 2009; Verbeke et al., 2014
      * Piessens 2011: caused 7.5% of clinical episodes of mastitis (7 total, 4 in mixed growth) – 2 of these were chromogenes (and these were single CNS growth infections)
    - **As a group, they can be persistent**
      * Some NAS infections can persist for an entire lactation
        + Aarestrup and Jensen, 1997; Chaffer et al., 1999; Taponen et al., 2007
      * Nyman et al 2018, in Sweden - up to 33% of quarters can be persistently infected with an NAS IMI
      * Valckenier 2021
        + 19.7% of 142 NAS IMI episodes were persistent
      * Piessens 2011
        + Up to 329.8 days (chromogenes), another quarter persistently infected with chromogenes during first lactation for 134.2 d and a second chromogenes during next lactation for 102.5 days (same quarter); 5 other persistent IMI, 63.2 to 290.5 days for chromogenes
        + Haemolyticus only one found to cause both transient and persistent infections
        + 84 total NAS IMI, 80/84 were persistent!
      * ***But, also shown that percentage have high spontaneous cure rate***
        + Spontaneous **cure rate** of untreated subclinical NAS IMI in first lactation (39.5% Taponen at al 2007) and older animals (64.5 % McDougall 1998, 72% Wilson et al 1999)
        + Valckenier 2020: 70.6% spontaneous cure rate of NAS infection in heifers during early lactation
    - **As a group generally, they do increase SCC**
      * Supre 2011
      * Fry 2014
      * Condas 2017 (quarter, SCC stuff)
        + Large number of samples from Canada (5,507 NAS isolates)
        + NAS IMI (as species group) increases SCC (70,000 cells/mL) significantly above uninfected quarters (32,000 cells/mL) but lower than major pathogen infected quarters (129,000-183,000 cells/mL)
        + Low SCC = 7.6% quarters infected with NAS, High SCC = 18.5% infected with NAS, Clinical mastitis 4.3% NAS
      * Wuytack 2020 (November JDS)
        + NAS quarters SCC higher than uninfected and Corynebac- and no different than those with MAJOR pathogens. "The NAS-positive milk samples (all species combined) had a significantly higher LnqSCC than the milk samples yielding no growth (P = 0.000) and Corynebacterium spp. (P = 0.008), yet the LnqSCC was not different from the major pathogen–positive milk samples."
      * Valckenier 2019
        + Non-aureus staphylococcus ***(all NAS, as a group)***IMI at 1 to 4 DIM was found to cause a slight but significant increase in qSCC (89,000 cells/mL) during the first 130 DIM compared with that in noninfected quarters (66,000 cells/mL)
        + Milk from quarters infected with NAS had a significantly higher sampling day qLnSCC than noninfected quarters (LSM = 4.49 and 4.19, respectively; P < 0.001) and a not statistically significant lower sampling-day qLnSCC than milk from quarters infected with a major pathogen (LSM = 4.94; P = 0.14).
      * Valckenier 2020
        + Persistently NAS (all)-infected quarters in the first 18 days had highest qSCC later in lactation (226,000 cells/mL infected with same NAS at BOTH first and second sampling), followed by quarters with a new NAS (all) IMI (119,000 cells/mL, IMI first identified at SECOND SAMPLING)

**No elevation in SCC for quarters only transiently infected with NAS (all) at first sampling** (+ at first, - at second) – not significantly different in later lactation than uninfected quarters (73,000 for transient, 63,000 uninfected)

* + - * Tomazi 2015
        + Compared quarters within cow, one infected with NAS and another not infected with NAS
        + Generally, for all CNS, milk yield and composition were unaffected but **quarter SCC was elevated** (306,106 cells/mL vs. 62,807 cells/mL, n=41)
    - **Effect of subclinical NAS IMI on milk yield**
      * *Potentially negative effects on milk yield, as summarized by Valckenier 2019* 
        + Timms and Schultz 1987
        + Grohn at al 2004
        + Taponen 2006
      * *Only slightly increase SCC but do not affect milk yield, as summarized by Valckenier 2019* 
        + Paradis 2010
        + Pearson 2013
        + Tomazi 2015
      * Heikkila et al 2018
        + Negative effect of NAS, identified by PCR, on udder health and milk yield – very large, multiyear study in Finland
        + *“Clinical mastitis caused by the minor pathogens … NAS also had a negative effect on milk production: … 5.7% (1.8 kg/d) in NAS when … diagnosed before peak lactation. In conclusion, minor pathogens should not be underestimated as a cause of milk yield reduction”*
      * Tomazi 2015
        + Compared quarters within cow, one infected with NAS and another not infected with NAS
        + Generally, for all CNS, milk yield and composition were unaffected but quarter SCC was elevated (306 106 cells/mL vs. 62,807 cells/mL, n=41)
      * Valckenier 2019
        + No significant difference in daily qMY was present between quarters infected with NAS IMI in early lactation and noninfected quarters during the first 4 months in lactation
      * Valckenier 2020
        + Average daily quarter milk yield in first 4 months of lactation did not differ between noninfected quarters and quarters infected with all other NAS at the first sampling day (or specifically staph chromogenes, for that matter)
        + The IMI status of quarters in the first 18 DIM, combining culture results at 1 to 4 and 15 to 18 DIM **(new, persistent, and transient IMI),** was not significantly associated with daily quarter milk yield in the first 4 months after calving.
        + **Both generally and taken as different “duration of infection types,” NAS (all) had no effect on milk yield**
* ***Chromogenes is of special concern (most widespread, most persistent, most increase in SCC)***
  + **Chromogenes most common when NAS speciated**
    - In a 2015 review summarizing work on NAS up to that point, Staph chromogenes was widely shown to be the most prevalent NAS species in milk samples isolated from both healthy primiparous and multiparous cows, and cows with subclinical or clinical mastitis in most countries (Vanderhaeghen et al, 2015).
    - Mork 2012 (32% of NAS)
    - Sampimon, 2011 (36% of NAS)
    - Gillespie 2009
    - Piessens 2011 (30.6% of all NAS isolates)
    - Condas 2017 (chromogenes most prevalent, all Canada prevalence study)
    - Valckenier 2020
      * Staphylococcus chromogenes was the most prevalent species on the first (29.4% of all NAS) and second (52.9% of all NAS) sampling days
    - Valckenier 2021
      * chromogenes most prevalent NAS identified over all isolates speciated over study at 52% of all NAS
    - Rowe 2019 (61% of all NAS)
    - Fry, 2014 (48% of all NAS)
    - Qu, 2019 (33% of all NAS)
    - Taponen 2007
    - Tomazi 2015 (74% of all NAS)
    - De Visscher 2016 (41.4% of all NAS)
      * Chromogenes is most universal (in 13 Flemish herds in fresh heifers and cows)
        + Only species found in all herds was chromogenes; otherwise, species distribution varied significantly by herd
    - Wuytack 2020 (November JDS)
      * 10% of ALL (not just NAS-positive) quartermilk samples were positive for chromogenes IMI
      * most prevalent species in “healthy” quarters or quarters with subclinical signs, and one of the 3 most commonly isolated species from quarters showing clinical signs
    - Person Waller, 2011 – chromogenes *second* most common, after epidermidis
  + **Chromogenes can be persistent** 
    - Supre 2011 (chromogenes in a group of NAS that can be persistent)
      * WHOA didn’t use any strain typing to confirm persistent infections
    - Piessens 2011 (chromogenes in a group of NAS that can be persistent)
      * 80/84 NAS infections were persistent, and 35/80 persistent infections were due to chromogenes (155.7 days on avg)
      * *However,* kind of biased by their definition of IMI: needed to be positive 2/3 months in a row; say they underestimated IMI, esp. short duration
      * *Chromogenes caused the MOST and the LONGEST persistent subclinical IMI*
      * AFLP for strain typing (amplified fragment-length polymorphism)
    - Thorberg, 2009 (chromogenes in a group of NAS that can be persistent)
    - Fry 2014 (chromogenes in a group of NAS that can be persistent)
      * PFGE
    - Mork 2012 (chromogenes in a group of NAS that can be persistent)
    - Nyman 2018 – species-specific differences in persistence capacity – chromogenes was third most persistent
      * In those quarters with Staph chromogenes specifically, almost 40% of infections were persistent
    - Valckenier 2021 – **chromogenes specifically more persistent than other NAS** (110 days vs. 70 days for other species)
      * 18/40 IMI with chromogenes (45%) persisted over at least two sampling periods, compared to 10/102 (9.8%) of IMI due to other species
      * Used RAPD to confirm persistency
    - *Actually, found NO species difference in persistence: Bexiga 2014*
  + **Chromogenes increases SCC**
    - Condas 2017 (quarters, SCC)
      * Chromogenes was the most frequent cause of CLINICAL NAS mastitis (similar to Zadoks and Watts 2009); others have reported simulans to be most frequent cause of clinical mastitis from NAS
    - Piessens 2011: caused 7.5% of clinical episodes of mastitis (7 total, 4 in mixed growth) – 2/3 pure NAS clinical were chromogenes
    - Tomazi 2015 – chromogenes increases quarter SCC
      * Could only do chromogenes when comparing qSCC to neg quarters
    - De Visscher 2016 – (one of three that elevates SCC)
      * Groups chromogenes in with simulans and xylosus, doesn’t have individual species estimates
    - Supré, 2011: one of a few species that are “more relevant for udder health;” because they can increase quarter SCC to a level comparable to that of S. aureus
    - Fry 2014: (one of few that elevates SCC)
    - Valckenier 2020:
      * Groups into “chromogenes” and “non-chromogenes” for SCC comparisons
      * *“we concluded that IMI with S. chromogenes starting in the first 18 DIM resulted in a significantly higher qSCC during the first 130 DIM, whereas IMI with the group of other NAS species had no effect on qSCC”*
      * Quarters infected with Staph. chromogenes at the first sampling day had a significantly higher qSCC in later lactation than noninfected quarters, whereas this was not true for quarters infected with all other NAS species (i.e., as a group of species). ***When you pull out chromogenes infections from the group “ALL NAS,” “ALL NAS” no longer caused an elevation in SCC (as was seen in 2019 paper)!***
      * ***First sampling day:***
        + Quarters infected with Staph. **chromogenes** at the first sampling day (n = 20; 1–4 DIM) had a **significantly higher** sampling day qLnSCC **than noninfected quarters** (n = 220; 144,000 cells/mL vs. 67,000 cells/mL, respectively; P < 0.001), and a sampling day qLnSCC that did not differ from quarters infected with a major pathogen (n = 9; 162,000 cells/mL; P = 0.99)
        + **No difference between** **SCC in quarters infected with Staph. chromogenes and quarters infected with a major pathogen**
        + **No difference between quarters infected with any other NAS species than Staph. chromogenes** **and noninfected quarters**
      * ***Overall, by persistence type, all chromogenes increased qSCC***:
        + Transient chromogenes (93,000 cells/mL, n=12), new chromogenes (302,000 cells/mL, n=5) and persistent chromogenes (340,000 cells/mL n=8) all significantly higher sampling day SCC vs. uninfected quarters (63,000 cells/mL, n=177)
        + No statistical difference between new chromogenes and persistent chromogenes SCC
      * Staphylococcus chromogenes caused an increase in qSCC comparable to the level of quarters infected with a major pathogen
    - Valckenier 2021:
      * Groups into “chromogenes” and “non-chromogenes” for SCC comparisons
      * Changes in quarter-level SCC, major pathogens vs. chromogenes vs. all other NAS combined as a group
      * ***Persistent infections:***
        + **pIMI with S. chromogenes 351,000** cells/mL, **pIMI with NAS “other than S. chromogenes” 213,000** cells/mL, **pIMI with a major pathogen 659,000** cells/mL; all these significantly higher than **noninfected** quarters **56,000** cells/mL
        + qSCC persistent **chromogenes not significantly different than quarters with a persistent infection with a MAJOR PATHOGEN**
        + Persistent major path vs. persistent chrom? Not different. Persistent major path vs. persistent “all other NAS”? Different.
        + **Persistent chrom vs. persistent “all other NAS” = not different**
      * ***Transient infections***
        + qSCC of transiently infected chromogenes (69,000 cells/mL) not different than uninfected quarters (56,000 cells/mL); qSCC of transiently infected quarters with “other” NAS as a group and transient infections with a major pathogen higher than uninfected quarters (small, but significant elevation)
    - Wuytack 2020 (November JDS)
      * most prevalent species in healthy quarters or quarters with subclinical signs, and one of the 3 most commonly isolated species from quarters showing clinical signs
      * only has qSCC for chromogenes and haemolyticus
      * Chromogenes and HEM SCC: The geometric mean qSCC of samples positive for S. chromogenes (n = 20) or S. haemolyticus (n = 10) only were 156,000 cells/mL of milk (IQR 81,000 to 212,000 cells/mL of milk) and 177,000 cells/mL of milk (IQR from 78,000 to 238,000 cells/mL of milk), respectively. **These milk samples had a higher LnqSCC than bacteriologically negative milk samples** (P = 0.000 and P = 0.001, respectively) and samples harboring Corynebacterium spp. (P = 0.008 and P = 0.049, respectively), **yet the LnqSCC was not different from the major pathogen–positive milk samples**.
    - Wuytack 2020 (March Vet Research)
      * Chromogenes IS more of a concern; in one of their herds, *“only samples from quarters with a SCC >50 000 cells/mL milk and clinical signs harbored S. chromogenes;” was the sole reason for increased SCC and clinical mastitis on this particular herd*
  + **Risk factor for developing IMI with S. aureus**
    - Reyher 2012
      * the presence of CNS two samplings before the occurrence of a new IMI increased the odds of experiencing a new Staph. aureus IMI by a factor of two
  + **No effect on milk yield**
    - Valckenier 2020
      * Average daily quarter milk yield in first 4 months of lactation did not differ between uninfected quarters, and quarters infected with Staph chromogenes or all other NAS at the first sampling day
    - Valckenier 2021
      * No significant differences in qMY were observed between quarters having a pIMI or tIMI with S. chromogenes or with the other NAS species compared with noninfected quarters
      * **BUT,** quarters that cured from an IMI with S. chromogenes had a significantly lower qMY than noninfected quarters
        + ***How is a “cure” different than a “transient” infection?***

***Transient = [pos] -> [neg] -> [neg - CURRENT SAMPLING DAY]***

***Cured = [pos] -> [neg – CURRENT SAMPLING DAY] -> neg***

* + - Tomazi 2015
      * Compared quarters within cow, one infected with NAS and another not infected with NAS
      * Generally, for all CNS, milk yield and composition were unaffected but quarter SCC was elevated (306, 106 cells/mL vs. 62,807 cells/mL, n=41)
      * Subclinical IMI caused by chromogenes had no effect on quarter milk yield or composition (despite increased qSCC) in both first and older lactation cows; measured at one point in time (no longitudinal follow-up; only chromogenes had high enough sample size for within-cow comparison)
  + **Even if doesn’t affect milk yield per se, DOES cause elevation in SCC** – and when infections are widespread within a herd, this slight increase in SCC multiplied over the whole herd can have real effects on BTSCC, milk quality measurements and producer compensation
    - High prevalence of CNS infections with relatively low SCC might significantly contribute to BTSCC (Rainard et al., 1990; Davidson et al., 1992; Piepers et al., 2009; Sampimon et al., 2009a; Schukken et al., 2009).
* **CNS heterogeneity, factors predicting variation**
  + ***NAS diversity in general***
    - … **“although the distribution of species has been shown to greatly differ between herds”**
      * Cited: De Visscher 2016 , Condas 2017 - Distribution of non-aureus staphylococci species in udder quarters with low and high somatic cell count, and clinical mastitis, Dolder 2017, Mahmmod 2018
      * *From Valckenier 2021, look into these for how risk factors at the herd, cow, and quarter level have been identified for multiple species*
        + *Piessens et al., 2011 – Distribution of coagulase-negative Staphylococcus species from milk and environment of dairy cows differs between herds*
        + *Bexiga et al., 2014 - Dynamics of bovine intramammary infections due to coagulase-negative staphylococci on four farms*
        + *De Visscher et al., 2016 - Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health*
    - Effect of NAS generally varies with **multiple factors**, Valckenier 2020
      * *The effect of NAS IMI at the first sampling day on qSCC in later lactation depends on the NAS species, whether the infections persists for at least 2 wk, and the time after calving when infection occurs (1–4 DIM vs. 15–18 DIM*
    - NAS species distribution varies due to multiple factors
      * Dolder 2017
        + **Chromogenes**: risk factors were being a cow in herd B, time period from June 2014 to August 2014, and December 2014 to February 2015, and udder edema
        + **Haemolyticus***:* risk factors were coinfection with xylosus, time period June 2014 to November 2014
        + **Xylosus:** Coinfection with haemolyticus and other staphs, June 2014 to August 2014, December 2014 to February 2015, early stage of lactation (first 60 days in milk), and belonging to herd B
        + **Warneri:** Mid and late lactation, coinfection with xylosus, September 2014 to May 2015 (cooler weather); herd-specific pattern most pronounced for warneri
    - Variation in species diversity herd to herd, prevalence of NAS herd to herd
      * *Bexiga et al., 2014 - Dynamics of bovine intramammary infections due to coagulase-negative staphylococci on four farms*
        + Species diversity varied between 4 farms studied
      * Mahmmod 2018
        + Species distribution of NAS differed between 8 study herds, in both milk samples and teat skin

P.S. only chose ELEVATED SCC milk samples, so biases what kind of NAS you’re going to find

* + - * + Isolating chromogenes on teat skin didn’t put quarter at elevated risk of chromogenes IMI
      * Wuytack 2020 (November JDS)
        + 33% of the quarter milk samples were NAS-positive (range 18 to 50% per herd), harboring 22 NAS species (range 5 to 15 species per herd)
        + Diverse range between herds for how much NAS were responsible for clinical mastitis

0% in one herd, 25% of clinical cases in another

Could likely be explained by variation of species/strain diversity between herds, and variation of virulence potential of both various species/strains

* + - **NAS species differ in their probable source (environment vs. intramammary)** 
      * Piessens 2011
        + Species predominating in the **environment:**

Equorum, sciuri, haemolyticus, fleuretti

Haemolyticus and equorum from stall air

Sciuri and simulans from slatted floors and sawdust of cubicles; USED bedding, may indicated contamination by cows for simulans

**Xylosus and succinus from sawdust stock**

**Haemolyticus and simulans found in the environment**; suggesting IMI from these was environmental in origin

Similar AFLP types of haemolyticus observed among isolates from environment, and IMI, indicating env source for this IMI (was also on teat skin)

* + - * + **S. chromogenes and S. epidermidis** rarely found in environment; **predominantly found in milk; udder was the main reservoir**

Bc: rarely isolated from environment, IMI probably from cow’s skin, milker’s hands, other cows

* + - De Visscher 2016
      * Dirty teats: more likely cohnii, equorum, saprophyticus, sciuri (supports environmental nature of these species)
    - De Visscher 2017
      * Teat dipping decreased equorum (evidence it is from environment), and when higher levels of coliform contamination from environmental sources more likely to have equorum too
    - **NAS species differ in their potential to be persistent**
      * Supre 2011
        + S. chromogenes and S. xylosus cause more persistent than transient IMI, whereas other NAS species cause relatively more transient infections
      * Piessens 2011 (chromogenes in a group of NAS that can be persistent)
      * Thorberg, 2009 (chromogenes in a group of NAS that can be persistent)
      * Fry 2014 (chromogenes in a group of NAS that can be persistent)
      * Mork 2012 (chromogenes in a group of NAS that can be persistent)
      * Nyman 2018 – species-specific differences in persistence capacity – chromogenes was third most persistent
      * Valckenier 2021 – **chromogenes specifically more persistent than other NAS**
      * *Actually, found NO species difference: Bexiga 2014*
    - **NAS species differ in their effect on milk yield**
      * Thorberg 2009 - species-specific effects on milk yield are linked with persistence of IMI
        + nonpersistent IMI due to Staph. epidermidis lead to significantly lower MY than persistent Staph. chromogenes or Staph. simulans IMI, whereas nonpersistent IMI due to Staph. simulans lead to significantly lower MY compared with healthy cows or cows with persistent IMI due to Staph. chromogenes, Staph. epidermidis, or Staph. simulans.
    - **NAS species differ in potential to increase qSCC** 
      * Condas 2017 (quarter level, SCC)
        + SCC highest (of all NAS quarters) for quarters with capitis, gallinarum, hyicus, agnetis, simulans
        + SCC lowest (of all NAS quarters) for xylosus, cohnii, equorum
        + Clinical cases – most frequently isolated sciuri
        + Chromogenes, simulans, xylosus, haemolyticus, epidermidis, agnetis, arlettae, capitis, gallinarum, sciuri, warneri more prevalent in high than low SCC quarters
        + **Distribution of different NAS species differed between high and low SCC quarters, AND different NAS species had diverse effects on SCC**
        + **S. chromogenes, S. simulans, and S. sciuri were the species most frequently isolated from clinical mastitis**
        + **S. agnetis, S. capitis, S. hyicus, S. gallinarum, and S. simulans increased SCC the most.**
      * Tomazi 2015 – chromogenes increases quarter SCC
      * De Visscher 2016
        + Chromogenes, xylosus, simulans associated with higher cell count at parturition vs. other species found (Flemish dairy herds,
      * Supré, 2011: chrom one of a few species that are “more relevant for udder health;” because they can increase quarter SCC to a level comparable to that of S. aureus; (group of NAS that can be persistent: chromogenes, simulans, xylosus)
      * Fry 2014: (chrom one of few that elevates SCC)
      * Valckenier 2020:
        + Quarters infected with Staph. chromogenes at the first sampling day had a significantly higher qSCC in later lactation than noninfected quarters, whereas this was not true for quarters infected with all other NAS species (chromogenes differs in *having ability* to increase SCC above normal, whereas group “all other NAS” do not – *note: no significant difference in direct comparison of SCC for quarters with “other NAS” IMI and chromogenes IMI, specifically)*
      * Wuytack 2020 (November JDS)
        + Chromogenes and HEM SCC: The geometric mean qSCC of samples positive for S. chromogenes (n = 20) or S. haemolyticus (n = 10) only were 156,000 cells/mL of milk (IQR 81,000 to 212,000 cells/mL of milk) and 177,000 cells/mL of milk (IQR from 78,000 to 238,000 cells/mL of milk)
      * Nyman 2018: species-specific effect on SCC
    - **NAS species differ in interaction with host immune response** 
      * Below studies, don’t lead to workable conclusions on species-specific difference on interaction with host/udder health, because of important differences in study design and investigated species – but can *hint* at NAS differing in interaction with host immune response
        + Taponen 2006 – simulans and chromogenes caused subclinical and clinical mastitis in equal proportion, ***no difference in severity between the species***
        + Hyvönen et al. 2009

**No pronounced differences between species**, instead observing large within-species variation **in adhesion and invasion capacity** for Staph. chromogenes, Staph. epidermidis, Staph. haemolyticus and Staph. simulans

* + - * + Persson Waller 2011 – no difference in proportion of clinical and subclinical cases for chromogenes, simulans, haemolyticus; epidermidis was more subclinical cases; higher proportion of clinical cases for hyicus than subclinical cases
        + Simojoki 2011 – experimental challenge with simulans elicited stronger immune response than epidermidis
        + Simojoki 2009 – 5 cows challenged with chromogenes strain all developed clinical mastitis in that quarter; but SCC and other indicators of inflammation (SAA, MAA) considerably lower than challenge with simulans and epidermidis; but can’t really compare, as inoculum dose different
        + Thorberg 2009 – epidermidis, simulans, chromogenes persistent infections induced mild to strong inflammatory response as judged by CMT, vs xylosus and haemolyticus had low CMT
      * Bryene 2015
        + Based on host immune response to the different strains in mice (differences in cytokine data, grouping of neutrophils) and the low prevalence in milk samples of cows for fleurettii…
        + S. fleurettii is able to colonize the milk habitat but is less likely to multiply locally compared with both S. chromogenes strains
      * Avall-Jääskeläinen, 2013
        + Bovine-associated CNS species resist phagocytosis differently
        + This study examined phagocytosis and killing by mouse macrophage cells of three CNS species: Staphylococcus chromogenes (15 isolates), Staphylococcus agnetis (6 isolates) and Staphylococcus simulans (15 isolates)
        + All the studied CNS species were phagocytosed by macrophages, but S. simulans resisted phagocytosis more effectively than the other CNS species. Only S. chromogenes was substantially killed by macrophages.
      * Mork 2012
        + “There seems to be strain differences… among CNS… with respect to transmissibility and pathogenicity.”
  + ***What kinds of factors can help explain NAS diversity?***
    - ***Geographical variation (country, region)***
      * In a 2015 review summarizing work on NAS up to that point, Staph chromogenes was widely shown to be the most prevalent NAS species in milk samples isolated from both healthy primiparous and multiparous cows, and cows with subclinical or clinical mastitis (Vanderhaeghen et al, 2015).
      * However, some **geographical variation** exists when considering the dominant NAS species. Studies in Sweden found Staph epidermidis to be the most frequently isolated NAS from cases of subclinical mastitis (Person Waller et al 2011, Nyman et al 2018), whereas Staph simulans was the most common species found in Finland (Taponen et al, 2016).
      * Distribution of NAS species differed among 4 regions of Canada in Condas 2017 (all Canada prevalence paper)
    - ***Seasonal effect***
      * De Visscher 2017
        + Seasonal effect observed for several species
      * Dolder 2017
        + Certain time periods were riskier for chromogenes, haemolyticus, warnerii, xylosus
    - ***Herd-level variation***
      * Condas 2017 (Canada prevalence)
        + **Facilities:**

Overall prevalence of NAS similar across barn types

91 herds, 60% tiestall, 33% freestall, 6% (n=5) bedded pack; excluded herds with mixed barn design

Simulans, xylosus, cohnii, saprophyticus, capitis, arlettae IMI higher in tiestall barns; prevalence of epidermidis was lowest

**Chromogenes and sciuri highest in bedded pack barns**

**High prevalence of xylosus and simulans could be linked to sawdust, which is mostly used for bedding in stalls in tiestall and freestall herds**

* + - * + BTSCC

Simulans, epidermidis, xylosus, cohnii more prevalent in herds with intermediate to high BTSCC, haemolyticus most prevalent in high BTSCC herds, other species no difference in distribution

* + - * De Visscher 2017 (BULK TANK MILK): *Coagulase-negative Staphylococcus species in bulk milk: Prevalence, distribution, and associated subgroup- and species-specific risk factors*
        + Identification of knowledge gap:

*“A huge variation in species distribution among herds has been observed in several studies, emphasizing the need to identify subgroup- and species-specific herd-level factors to improve our understanding of the differences in ecological and epidemiological nature between species.”*

*“Describing CNS prevalence and distribution in those habitats and identifying associated risk factors, as has been done for other mastitis pathogens, is the obvious next step toward a better understanding of the variation in epidemiological and ecological nature among species”*

* + - * + Difference in prevalence of CNS associated with **facility type**

Loose packs or tiestalls more likely to yield CNS than freestalls (except epidermidis, simulans, cohnii- these three did not vary either bc they are just so ubiquitous, or bc their presence in BTM is more cow-dependent)

“Housing of lactating animals most likely also plays an important role in our study, as the presence of only 3 species was not associated with this risk factor”

* + - * + Management factors

In September, herds with **udders clipped** had lower odds of yielding chromogenes, simulans, xylosus (species more relevant for udder health)

Herds participating in monthly veterinary udder health monitoring program more likely to yield these 3 species (that are more relevant to udder health)

Likely reverse causation – these 3 species increase SCC and are persistent, so herds with higher number of cows with elevated SCC decided to monitor udder health more closely with their vet

Herds that always received milk quality premium (most common cause for not receiving = coliform increase, and equorum is environmental in nature, so this makes sense), or herds that predisinfected teats before attaching milking cluster (also supports environmental nature of equorum) less likely to have equorum in BTM

Herds not using single dry cotton or paper towel for each cow more likely to have cohnii

Cohnii may be in teat liners, on teat skin, or coming from cows with SCIMI so hard to pinpoint if more env or more udder

Herds that flushed milking units/steamed them after cow with SCIMI milked less likely to have haemolyticus, simulans, cohnii

Haemolyticus and simulans, likely source is from milk (from SCIMI cows)

Always wearing gloves during milking decreased devriesei

Tap water used for drinking from public supply increased odds of simulans

* + - * Piessens 2011
        + Saw herd to herd differences in observed distribution of NAS species in BOTH environment and in milk; “herd-level factors are involved in the establishment of particular species in a dairy herd.”
        + Primary reservoirs of species causing IMI different herd to herd; but herds were similar in management and farm characteristics, so not really able to tease apart why they may have seen these differences
      * Passych 2014
        + Supplementation with vitamins, ease of calving associated with likelihood of CNS IMI in heifers (from De Visscher 2016, not important in 2016 study)
      * Piepers 2011
        + Teat dipping before parturition associated with likelihood of CNS IMI in heifers (from De Visscher 2016, not important in 2016 study) – no teat dip, unclipped udder, and poor hygiene increased odds of CNS IMI for heifers
        + Housing can influence HYGIENE, which is associated with increasing odds of CNS IMI in heifers at parturition
      * Dufour 2012
        + *Epidemiology of coagulase-negative staphylococci intramammary infection in dairy cattle and the effect of bacteriological culture misclassification*
        + No difference in prevalence of NAS IMI as a group, among tiestall, freestall, bedded pack herds
        + Lower CNS prevalence in Canadian herds with sand or wood-based bedding; and in herds where cows had access to pasture
        + ***Sand and wood-based product bedding showed desirable associations with CNS IMI compared with straw bedding.***
      * Sampimon 2009
        + Pasturing during the outdoor season associated with likelihood of CNS IMI in heifers (from De Visscher 2016, not important during 2016 study)
        + Dry cows housed in one group vs multiple groups, using non-tap water as drinking source, and lots of stalls contaminated with milk leakage increased CNS prevalence
      * Hogan et al 1987
        + CNS species distribution affected by type of germicide used for teat dipping
      * Jayarao 2004
        + Premilking teat disinfection lowered likelihood of finding CNS in BTM
      * White 1989
        + Type of housing affects CNS distribution on body sites
      * Matos 1991
        + Type of bedding affects type of CNS found… in BEDDING
      * *From Wuytack 2020 JDS:*
        + ***Herd-specific factors (e.g., housing conditions, clipping of the udder, and milking protocol) also influence species-specific prevalence as well as seasonal effects, although more research is required to identify species-specific risk factors***

***Cited De Visscher et al., 2016a, De Visscher et al., 2017, Dolder et al., 2017***

* + - * De Visscher et al., 2016a *- Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health*
        + Only species found in all herds was chromogenes; otherwise, species distribution varied by herd; “large between-herd differences in distribution were observed for the other species”
        + Variation existed both between cows, and between herds, for species considered “environmental” in nature; suggesting variation associated with management differences between herds (but again, most herds pretty similar in management so couldn’t make any direct correlations)
      * Dolder et al., 2017 ***-*** *Quarter- and cow-level risk factors for intramammary infection with coagulase-negative staphylococci species in Swiss dairy cows*
    - ***Animal-level factors***
      * ***Udder edema***
        + Dolder 2017

Risk factor for chromogenes

Likely, decreased blood circulation and poor conformation to milking units decreases udder defense mechanisms

* + - * ***Anatomical variation/cleanliness of teat skin***
        + Teat location?

Barkema 1997 found more NAS in rear than front quarters, but De Visscher 2016 did not report species-specific NAS quarter distribution

* + - * + De Visscher 2016

Quarters with an inverted teat end had higher odds of being infected with chromogenes, simulans, or xylosus as well as with chromogenes solely

Inverted = milk deposits on teat end, good growth substrate for bacteria and larger streak canal also associated with this trait (easier access for bacteria)

Prepartum teat skin colonization with chromogenes increased likelihood of chromogenes IMI in corresponding quarters at parturition

Dirty teats: more likely cohnii, equorum, saprophyticus, sciuri (supports environmental nature of these species)

* + - * ***Parity***
        + ***Prevalence can vary by parity***

Heifers more likely to have an IMI due to NAS pre-freshening or during the fresh period when compared to multiparous cows

Taponen et al 2007 – 37.5% of quarters vs. 5.8% of quarters infected with NAS for heifers vs. multiparous cows

Oliver et al 2003 – up to 45.5% quarters might have NAS at first calving (heifer calving in first time)

Condas 2017 (all Canada prevalence)

Prevalence of NAS highest in first-parity heifers, evenly distributed throughout cows in second lactation and up

Heifers and older cows more frequently had NAS

Nyman et al 2018- of all NAS infected milk samples, 38% were IMI from heifers, 26% second lactation, and 36% older than second lactation cows

* + - * + ***Species distribution can vary by parity***

Staph chromogenes, Staph xylosus, Staph simulans more commonly found in heifers than third-parity and older cows

Thorberg et al., 2009; De Visscher et al., 2016; Condas et al., 2017a (Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds); Nyman et al., 2018

Also found this in De Visscher 2016: heifers more likely to have these three species, or chromogenes solely

Mork 2012 found chromogenes more in primiparous animals, epidermidis more in multiparous animals

* + - * ***Stage of lactation***
        + ***Species distribution can vary with stage of lactation***

Condas et al 2017 (Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds)

* + - * + In Flanders, Staph chromogenes predominant species both at freshening and throughout lactation in randomly selected heifers and older cows; followed by Staph sciuri and Staph cohnii at parturition (De Visscher 2016); then Staph simulans, Staph xylosus, Staph epidermidis, Staph haemolyticus during lactation (Piessens et al 2011, Supre et al 2011)
* ***Diversity within NAS species, and chromogenes in particular***
  + Summary from Vanderhaegen **2015**, CNS review article, on the ecology and epidemiology of CNS (chromogenes)
    - *High levels of diversity among S. chromogenes from bovine IMI have been detected by AFLP (Taponen et al., 2006) and PFGE (Gillespie et al., 2009; Rajala-Schultz et al., 2009). Post-milking teat dipping has little effect on S. chromogenes (Quirk et al., 2012). Overall, this suggests that S. chromogenes originates from a nearby extra-mammary source and that most IMI due to this bacterium are opportunistic. S. chromogenes IMI may also be contagious, since some S. chromogenes strains have been found in multiple animals from the same farm (Taponen et al., 2008; Gillespie et al., 2009; Mørk et al., 2012). However, it is not clear whether these observations could be explained by spread through a vector. S. chromogenes was identified in milk samples from 18 cows on three farms in Belgium, but was absent from milking machine unit liners and human skin/gloves, which are considered to be the most important routes of transmission for contagious mastitis (De Visscher et al., 2014). Piessens et al. (2011, 2012) concluded that cows were the main source of S. chromogenes isolated from milk (i.e. that the bacterium is an udder-adapted species), since S. chromogenes was identified more frequently from milk than environmental samples, and several environmental strains had similar AFLP and RAPD profiles to those in milk (suggesting that S. chromogenes was present in the environment as a result of ‘contamination’ from spilled milk). However, Braem et al. (2013) were unable to detect S. chromogenes on the teat apices of these cows by culture or culture-independent methods (although no other body sites were investigated). In other studies, S. chromogenes has been detected in teat canals, on teat skin and other body sites (White et al., 1989; Trinidad et al., 1990; Matthews et al., 1992). S. chromogenes originating from milk had PFGE profiles that corresponded to strains isolated from the teat canal, teat apex, udder skin and perineum, but the direction of transmission could not be determined (Taponen et al., 2008). Thus, it appears that S. chromogenes is a host-adapted species, commonly inhabiting bovine skin, but it is uncertain whether it predominates in any specific region or inhabits internal udder tissue.*
  + ***Evidence for within-species variation amongst NAS generally***
    - Mahmmod 2018
      * Some species of NAS differed between isolates of same strain in interaction with S. aureus, when they were looking at cross-talk between NAS and SA/quorum sensing
    - Condas 2017 (quarters, SCC)
      * Evidence for strain diversity
        + **Chromogenes, simulans, epidermidis, haemolyticus isolated with similar frequency from among low SCC and high SCC quarters and clinical mastitis cases**
    - Dolder 2017
      * Within warnerii on particular herd, seemed more contagious than warnerii in other herds
    - Fry 2014
      * Within a given species of NAS, considerable variation was observed for effect on SCC
    - Mork 2012
      * “It was shown that several pulsotypes (PTs) within each [NAS] species were associated with persistent infections, but only a few were spread and caused persistent IMI in multiple cows within a herd. Of special interest was the observation that only one, or a few, strains of each species caused persistent IMI in multiple cows within a same herd. This indicates strain differences with respect to transmissibility and pathogenicity”
    - Hyvönen et al. 2009
      * **No pronounced differences between species**, instead observing large within-species variation **in adhesion and invasion capacity** for Staph. chromogenes, Staph. epidermidis, Staph. haemolyticus and Staph. simulans
    - Avall-Jääskeläinen, 2013
      * Significant variations between isolates were seen in both phagocytosis and killing by macrophages and were more common in the killing assays
        + Isolate differences in phagocytosis were seen in S. simulans and in killing for all the studied species.
        + Suggesting possible strain difference in pathogenicity
    - Wuytack 2020 (November JDS)
      * *mecA was found in 49% of the NAS isolates originating from clinical mastitis and only in one isolate (6%) originating from healthy quarters. Although the presence of certain antimicrobial genes may be associated with reduced fitness of the strain, the methicillin-resistance gene, mecA in bovine NAS isolates might be linked with virulence genes or pathogenicity islands, supposedly both present on the mobile element, SCCmec.*
    - Thorberg 2009 - species-specific effects on milk yield are linked with persistence of IMI
      * In general, a significantly higher milk production was observed among persistently infected compared with nonpersistently infected cows and among healthy compared with nonpersistently infected cows.
      * Within a species, could exist different transient and persistent strains, which varied also in their effect on milk yield
    - From Vanderhaeghen review*:*
      * *Persistence is also partly dependent on host-microbe interactions and the quality of the host’s immunity; certain strains were found to cause persistent IMI in some cows and transient IMI in others* 
        + Taponen et al., 2007; Simojoki et al., 2009, 2011; Piessens et al., 2011
      * *It appears that persistence is partly strain dependent, with several CNS species comprising strains that do cause and others that do not cause persistent IMI*
        + Gillespie et al., 2009; Piessens et al., 2011; Mørk et al., 2012
      * *whether the strains from a given species that cause or do not cause IMI across studies have a similar genetic background remains to be elucidated, as this requires using typing methods allowing for largescale comparisons, such as multilocus sequence typing, which has not been done so far.*
  + ***Some studies found more genetic diversity amongst chromogenes, some less***
    - Mostly heterogenous
      * Sheela 2021: Four predominant RAPD groups among 37 isolates in India
      * Qu 2018: eight distinct RAPD types observed, with 73% of the isolates being represented by 2 most prevalent types in China on one farm
      * Gillespie 2009: 66 S. chromogenes isolates obtained from 30 animals from three dairy herds of the USA, where 33 different PFGE patterns were observed indicating genetic diversity in strains even when multiple sampling was done
      * Wuytack 2020 (March Vet Research)
        + *"Among the 22 S. chromogenes isolates, originating from herd 1 only, 7 different fingerprints (a-g, 60% similarity) were identified (Figure 1). Five RAPD types (a, b, c, d, and f) were identified more than once. RAPD type d (n= 6) was isolated from all the habitats, i.e. quarter milk (as cause of both subclinical and clinical mastitis, n= 4), teat apices (n= 1), and rectal feces (n= 1). On the other hand, type f was only detected in milk, as cause of both sub-clinical and clinical mastitis. In cow F, multiple isolates of S. chromogenes (n= 4) were found in the same quarter (clinical mastitis) over a time period of 32 days, belonging to 3 different RAPD types”*
    - Mostly conserved amongst chromogenes
      * Shimizu 1997: PFGE, some bovine isolates, “highly conserved”
      * Piessens 2012: (n=28) bovine IMI showed limited genotypic heterogeneity by RAPD both within and between Flemish dairy herds, reflecting high genetic conservation within the species.
    - Can’t tell if this is conserved or diverse
      * Mello 2020
        + By PFGE, characterized 27 chromogenes isolates, three clusters were formed. Don’t know if this is significant or not
    - Also, variable ways of characterizing strain types
      * Difficult to generalize the results or compare the RAPD or PFGE types between distant geographical locations due to the small number of studies relating S. chromogenes
      * RAPD, PCR-based
      * MLST
      * **Combining MLST and WGS would be cool**
        + **Wuytack 2020 (March Vet Research)**

**The low mutation rate of housekeeping genes makes multilocus sequence typing more suitable, but it is currently only available for S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, and S. pseudintermedius. Whole genome sequencing provides a theoretically optimal resolution, but is, momentarily, less suitable for larger collections of isolates and routine analyses**

* + ***Evidence for existence of strain types within chromogenes specifically***
    - Valckenier 2021:
      * “The qSCC on sampling days where a quarter was considered cured from an IMI with S. chromogenes was still significantly higher (120,000 cells/mL) compared with that of noninfected quarters, which is remarkable given the fact that 16 of these 20 cIMI followed a tIMI. In quarters that cured from an IMI with NAS other than S. chromogenes or a major pathogen, qSCC was not different (63,000 cells/mL, P = 0.39; and 67,000 cells/mL, P = 0.27, respectively)
        + qSCC of transiently infected chromogenes not different than uninfected quarters; qSCC of transiently infected quarters with other NAS as a group and transient infections with a major pathogen higher than uninfected quarters
        + *Transient chromogenes IMI didn’t elevate above uninfected, but had some sort of lingering effect after a cure that was enough to elevate SCC?*
      * “regarding the ability to cause persistent infections, S. chromogenes seemed the best adapted to survive in the udder gland in our study”
        + Chromogenes more relevant for udder health, because in this study, qSCC for persistent chrom not different than qSCC for persistent major pathogen, AND large proportion of infections that persist for longer periods
      * Possibly, diversity within strain types that can differ in persistence or pathogenicity:
        + “we found about half of the IMI caused by S. chromogenes persisted compared with only 16.4% of IMI caused by the other NAS species. Even higher proportions of pIMI by S. chromogenes (69.6%) and other NAS (31.8%) have been reported (Supré et al., 2011).”

This half – does it constitute a separate strain type than the transient infections? *Does this argue for strain variation?*

* + - * + “Differences in persistence of NAS IMI are thus not merely the result of variations in study design and occurrence of certain species, but can vary between different strains of a NAS species (Piccart 2016)
      * **INDENTIFIES KNOWLEDGE GAP:**
        + **“For S. chromogenes, further investigation is needed to determine whether the strains that cause tIMI without elevated qSCC are different from the strains causing pIMI with a significantly higher qSCC or that other factors, such as the immunity of the quarter or animal, play a role.”**
    - Piccart 2016
      * Variability within chromogenes of host immune response
      * Experimental infection an intramammary Staphylococcus chromogenes strain originating from a persistent intramammary infection (S. chromogenes IM) and a S. chromogenes strain isolated from a heifer's teat apex (S. chromogenes TA). Bacterial and somatic cell counts, as well as neutrophil responses, were higher after inoculation with S. chromogenes IM than with S. chromogenes TA. In conclusion, these results suggest that S. chromogenes might be better adapted to the mammary gland than S. fleurettii. Furthermore, not all S. chromogenes strains induce the same local host response.
    - Breyne 2015
      * Results from mouse model suggest an underlying difference in the hosts’ innate immune response between S. chromogenes IM and S. chromogenes TA.
        + *More specifically, the S. chromogenes IM strain might be more adapted to the intramammary niche (milk environment) and as such induce less pathophysiological effects compared with the likely more teat-adapted S. chromogenes TA.*
    - Condas 2017 (quarter, SCC paper)
      * Half chromogenes isolates associated with high SCC infection, half with low
      * Potential here for strain variation!
    - Mork 2012
      * Of 56 persistent IMI, 20 caused by chromogenes
      * These 20 isolates belonged to 11 chromogenes pulsotypes, in 4 clusters
      * Isolates belonging to 5 of these pulsotypes were found to be persistent in *more than 1 quarter (contagious)*
        + Isolates in one pulsotype caused persistent infections in 3 cows in 1 herd
        + Isolates belonging to 2 other pulsotypes were found in persistently infected quarters in 2 cows in the same herd
  + ***Are different NAS strains more adapted to the udder?***
    - Dolder 2017
      * Summarizes how chromogenes likely behaves, ecologically
      * *The individual cow’s immune status as a potential risk factor was not assessed but might be relevant for species such as Staph. chromogenes and the Staph. warneri-like species where 26 and 21% of the variance, respectively, were attributable to the cow level. This corresponds well with the fact that Staph. chromogenes is a cow-associated mastitis pathogen with the udder as its reservoir but causes infection in an opportunistic way because it was found on teat and udder skin as well as in milk samples (Vanderhaeghen et al., 2015). A contagious behavior cannot be excluded because identical strains were found in several animals of one herd in other studies (Taponen et al., 2008; Mørk et al., 2012*
      * Found on teat skin – so, cow-adapted – most cows have it – but, variability mostly at cow-level – so, all cows have it on their body (and are “at risk”), but highly variable as to whether or not they get an IMI with it (suggests “opportunistic behavior” as pathogen) – this variability could likely be due to cow-immune status determinants **(OR devil’s advocate, is it different strain types…)**
    - De Visscher 2016
      * Teat apex colonization prepartum increased odds of chromogenes IMI at freshening- “illustrates host-adapted nature of chromogenes – teat apices might act as habitat for host-adapted species” (also mention Taponen 2008 has suggested chromogenes is host-adapted)
    - Piessens 2011 and 2012
      * *From De Visscher 2016:* Chromogenes most prevalent species causing IMI and is rarely isolated from bovine environment, strong evidence to show that “a host-adapted nature is assumed”
    - Valckenier 2020:
      * “Based on the ability to cause persistent infections and the genetic heterogeneity and clonality, some NAS species appear to be more adapted to the cow’s mammary gland, such as Staph chromogenes, Staph epidermidis, Staph simulans, and Staph hyicus, and might hypothetically have a different effect on qSCC and qMY than other less cow-adapted NAS species”
        + Taponen et al 2008, Piessens et al 2012, Fry et al 2014, Nyman et al 2018
    - Wuytack 2020 (March Vet Research)
      * *For S. chromogenes, S. devriesei, S. equorum, S. haemolyticus (herd 5), and S. hominis, one or more strains were solely found in either quarter milk or on teat apices suggesting some strains within species could be more udder-adapted while others have no or a lower ability to colonize the mammary gland. Strain differences within NAS species have actually been confirmed before. E.g. we reported that S. chromogenes originating from a persistent IMI displays a better in vitro bacterial growth in conditions mimicking the mammary gland and in vivo bacterial growth in the mammary glands of mice and dairy cows, compared to S. chromogenes isolated from the teat apex [9, 34, 35-Piccart 2016]. Differences in inflammatory response in both mice and dairy cows between strains of the same species and growth inhibition of other major mastitis pathogens have been demonstrated as well [35, 36],* ***indicating it is relevant to subtype NAS in future research efforts***
    - Breyne 2015
      * Compared host immune response induced by *S. chromogenes* IMI strain, *Staphylococcus fleurettii* from cow bedding sawdust, and *S. chromogenes* from a teat apex of a heifer
      * Epidemiologically different bovine CNS species or strains induce a differential host innate immune response in the murine mammary gland
      * Results from mouse model suggest an underlying difference in the hosts’ innate immune response between S. chromogenes IM and S. chromogenes TA.
        + *More specifically, the S. chromogenes IM strain might be more adapted to the intramammary niche (milk environment) and as such induce less pathophysiological effects compared with the likely more teat-adapted S. chromogenes TA.*
    - Souza 2016
      * *Interaction between bovine-associated coagulase-negative staphylococci species and strains and bovine mammary epithelial cells reflects differences in ecology and epidemiological behavior.*
* ***Linking strain type (genetic basis) and phenotypic trait (persistence, elevation of SCC, virulence factors)?***
  + ***Strain and virulence***
    - Virulence factors not yet found which can attribute persistence for CNS IMI (as of Vanderhaeghen review, 2014)
      * *It would be hypothesized that the presence of factors granting the ability to withstand the host’s immunity or external treatment will positively influence the capacity to persist in the udder.*
        + *No association between persistence of infection and biofilm formation in a tissue culture plate assay or a fluorescent in situ hybridization assay (Simojoki et al., 2012).*
        + *No difference in vitro adhesion to and invasion of bovine mammary epithelial cells when comparing strains originating from persistent and transient IMI (Hyvönen et al. 2009)*
  + ***Strain and persistence***
    - *An association between persistence and severity of IMI, measured through SCC, could not be established – as of Vanderhaeghen review 2014/2015*
      * + Taponen et al., 2007; Supré et al., 2011
    - Persistence type: no clear association with virulence factors or host immune response
      * Simojoki et al., 2011
        + In an experimental infection trial, no significant differences in various cytokines measured to look at immune response, or in SCC response could be demonstrated between persistent and transient IMI caused by either Staph. epidermidis or Staph. simulans
        + **BUT** in persistent IMI cases, concentrations of other cytokines usually associated with inflammation were lower, and in 2 persistent IMI cases, one (SAA) did not increase at all when challenged
        + These latter observations are in line with Simojoki 2009- experimental infection study with Staph. chromogenes persistent IMI lacked an inflammatory response
    - Thorberg 2009
      * Within a species, could exist different transient and persistent strains, which varied also in their effect on milk yield
  + ***Association between strain, virulence, clinical signs, antibiotic resistance carriage***
    - Wuytack 2020 (November JDS)
      * *The presence of virulence genes and the methicillin-resistance gene, mecA, dovetails nicely with the prevalence of NAS in the opposite strata of the quarter milk because NAS species were recovered 3 times as frequently in samples from clinical mastitis cases compared with milk samples from healthy quarters, suggesting that some NAS isolates have the ability to cause (mild) clinical mastitis.*
      * Might be some association with STRAIN TYPE (within species) being more pathogenic and presence of virulence, resistance
      * NAS and chromogenes certainly something to “worry” about; NAS can have the ability to cause mild clinical mastitis; because when comparing proportion of healthy and clinical milk samples that had ONLY NAS isolated, higher proportion of CLINCAL quarters had NAS (12%) than healthy quarters (4%)
    - Wuytack 2020 (March Vet Research)
      * Chromogenes IS more of a concern; in one of their herds, *“only samples from quarters with a SCC >50 000 cells/mL milk and clinical signs harbored S. chromogenes;” was the sole reason for increased SCC and clinical mastitis. “Also, the same strain was isolated from a quarter with subclinical mastitis and a quarter with clinical signs, substantiating that not only the virulence potential of the pathogen but also host factors might influence the severity of mastitis”*
* ***NAS possess virulence factors and genes for AMR***
  + ***Small ruminants?***
    - Martins KB et al. (2017)
    - Especially for beta-lactams, tetracyclines- commonly used to treat mastitis in animals
      * Onni T et al. (2011)
      * Turchi B et al. (2020)
  + ***Bovine mastitis***
    - Sheela 2021
      * 7 out of 14 bovine isolates methicillin resistant
    - Two Dutch studies found much lower percent methicillin resistance
      * Sawant, 2009
      * Fessler, 2010
  + ***Evidence of mechanisms for resistance in NAS***
    - Penicillin resistance
      * BlaZ encoding beta-lactamase
      * Penicillin-binding protein transpeptidase (PBP2a) encoded by mecA, carried on a mobile chromosomal element (Staphylococcal Chromosomal Cassette mec, SCCmec)
        + PBP2a homologue coded by mecC gene recent finding
      * Wuytack 2020 (November JDS)
        + prevalence of the mecA gene in NAS isolates from clinical mastitis cases was 49%, situated between the mecA prevalence of 29% described by Khazandi et al. (2018 – subset, only oxacillin resistant isolates tested) and the 95% reported by Mahato et al. (2017)
    - Antiseptic resistance genes (qacA/B, smr, qacG, qacH, qacJ)
      * Bjorland J et al. (2005)
      * Turchi B et al. (2020)
    - Resistance in chromogenes specifically
      * Lee 2020
        + MDR phenotype widespread among chromogenes isolates
        + 9 chromogenes isolates from retail chicken meat, all 9 were multi-drug resistant

Ampicillin

Penicillin

Chloramphenicol

Fluoroquinolone

Clindamycin

Erythromycin

Trimethoprim-sulfamethoxazole

Tetracycline

* + - * El-Ashek 2020
        + 14 buffalo/cow chromogenes IMI isolates

3/8 had blaZ and penicillin resistance

No MDR amongst chromogenes

Some isolates had cassette chromosome recombinase genes ccrA-1, while another isolate of cattle origin carried cassette chromosome recombinase genes “ccrAA” (hypothetical) and ccrC

Two cattle isolates and one from a buffalo harbored genes encoding beta-lactamase (blaZ), beta-lactamase repressor (inhibitor) (blaI), and beta-lactamase regulatory protein (blaR)

* + - * + Unlike some previous work, isolates of CoNS displayed a comparatively low prevalence of resistance genes
  + ***Mechanisms for virulence***
    - Staphylococcal enterotoxins
      * Lee 2020
        + Found in chromogenes isolates in retail chicken meat (multiple SE gene carriage often in chromogenes isolates)

Toxic shock syndrome toxin-1 gene

tst1

Classical staphylococcal enterotoxin genes

sea

Newer staphylococcal enterotoxin genes

seh, selj, sem, sep, sek, seo, sei

* + - Biofilms
      * From Vanderhaeghen 2014
        + Biofilm formation abilities appear not to be common in the CNS species found most frequently in milk
        + Staphylococcus chromogenes has not been identified as a superior biofilm producer or a very frequent biofilm gene carrier

Simojoki et al., 2012; Tremblay et al., 2013

* + - * + no association between biofilm formation and persistence of IMI.

Simojoki et al. (2012)

* + - * Mello 2020
        + Staph chromogenes IMI (27 isolates) did have some genes associated with making biofilms, although less than Staph epi and Staph aureus (icaABCD).

Even though genes were present, were not expressed really; Very limited expression of biofilm-producing genes in chromogenes isolates from variety of farms and geographical regions in Brazil

No chromogenes had bhp

* + - * + As a group, 32% NAS resistant to oxacillin – higher than S aureus
      * Wuytack 2020 (November JDS)
        + virulence and resistance factors they tested for presence of: agrA (accessory gene regulator protein), bap (biofilm-associated protein), cap5H and 8H (CP5 and CP8 synthesis enzyme), clfA (clumping factor), coa (coagulase), eno (laminin binding protein), fnbpA and B (fibronectin-binding protein A and B), hla (α-hemolysin), hlb (β-hemolysin), ica (polysaccharide intercellular adhesion), mecA and mecC (methicillin resistance), nuc (thermonuclease), ssl7 (staphylococcal superantigen-like protein), and vwb (von-Willebrand factor binding protein).
        + Relatively low prevalence overall of virulence genes in NAS identified by MALDI (had limited number of isolates ID’d with MALDI that had NAS only: n=134, and these 134 were divided over 15 NAS species, and 3 different milk strata – insufficient number for species-specific statistical analysis regarding virulence potential)
        + Virulence genes agrA, bap, and cap5H found in 44% of NAS isolates originating from quarters with clinical signs, vs. healthy quarter NAS only 19% harboring bap
        + All had eno (biofilm)
        + “All isolates tested positive for the biofilm-related gene, eno, a finding that is comparable to other studies that also reported a prevalence as high as 88% and 92.6%, respectively (Darwish and Asfour, 2013; Srednik et al., 2017). I
  + ***What kind of virulence genes do people even test for, and how?!***
    - **Biofilms**
      * *Mello 2020* 
        + Biofilms protects the bacteria against the action of immune system components by blocking phagocyte activity, and serve as a barrier that impairs the penetration of antimicrobial agents
        + Shown that *Staph. aureus* IMI isolates capable of creating a biofilm, and this may contribute to its ability to be a chronic, persistent intramammary infection and resist conventional antibiotic therapy; biofilms may be a selective advantage due to a strain characteristic

Aguilar 2001, Fox 2014

* + - * + *The proliferation of cells to adhere and form a biofilm is mediated by the production of polysaccharide intercellular adhesin (PIA). This adhesin is encoded by the gene product of the ica locus of the icaADBC operon, which is essential for biofilm formation and virulence of the microorganisms*

icaA, icaB, icaC, icaD

* + - * + *Another important gene that also regulates biofilm formation is biofilm-associated protein (bap), which encodes the bap surface protein. Unlike PIA which only seems to be involved in intercellular adhesion, this protein promotes primary binding to abiotic surfaces and intercellular adhesion. In addition to bap, the bhp gene is also related to biofilm formation irrespective of the presence of the ica operon*

bap, bhp

* + ***What kind of resistance genes do people even test for, and how?!***
    - **Generally, in staphylococci**
      * Methicillin resistance phenotype in staphylococci is mostly caused by the mecA gene that is located within a staphylococcal cassette chromosomemec (SCCmec)
        + MRSA phenotype is primarily associated with SEEmec

Rolo 2017

* + - * + Food-related MRSA demonstrated

Bhargava 2014

Yang 2017

* + - * Other mec genes, such as mecB and mecC, have also been recognized in association withβ-lactam resistance in staphylococci
        + Loncaric 2013
        + Becker 2018
      * mecB and mecC genes have usually been identified within mobile genetic elements (MGEs) that were similar to SCCmec
        + Gomex-Sanz 2015
      * **Several studies have reported that these SCCmec elements can be transferred between CoPS and CoNS isolates**
        + **Importance of CoNS and NAS as potential reservoirs of antimicrobial resistance genes**
        + Tsubakishita 2010
        + International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements 2009
        + Lee, 2019: *Transfer of a mobile Staphylococcus saprophyticus plasmid isolated from fermented seafood that confers tetracycline resistance*
    - **Antibiotic resistance**
      * *Longheu 2021*
        + Cefoxitin/oxacillin-resistant isolates were tested for carriage of mecA gene and SCCmec type by PCR. The presence of ccrAB5 and of the novel ccr allotype (ccrABSHP) was confirmed with published primer sets.

The chromosome cassette recombinases A and B, encoded by ccrAB genes located on SCCmec, play a key role in the excision of SCCmec

* + - * + Genes encoding resistance to penicillin (blaZ), macrolide (msrA, ermA, ermB and ermC) and tetracycline (tetO, tetK, tetM and tetL) were also identified by PCR
        + Genes encoding resistance to antiseptics (qacA/B and smr) were screened for by a multiplex PCR using published primer sets
* ***Importance for human health and One Health***
  + NAS are important causes of opportunistic infections in humans and animals and a potential cause of food poisoning
    - von Eiff C et al. (2002)
      * NAS causing disease in humans
    - Huebner, 1999
      * NAS causing disease in people and animals
  + Virulence genes found to cause disease in people and animals have been demonstrated specifically in NAS isolates from bovine IMI
    - Unal 2012
      * Staphylococcal enterotoxin, MRSA, Panton-Valentine leukocidin genes from cows and sheep with SCM
    - Orden 1992
      * Staphylococcal enterotoxin and TSST-1 (toxic shock) in bovine mastitis isolates
  + NAS have potential to serve as reservoirs of resistance genes, especially shared among Staphylococci
    - Especially those on transmissible plasmids (SCCmec)
      * **Several studies have reported that these SCCmec elements can be transferred between CoPS and CoNS isolates**
        + *Tsubakishita 2010*
        + *International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), Classification of staphylococcal cassette chromosome mec (SCCmec):* Guidelines for reporting novel SCCmec elements 2009
    - *de Freitas 2013*: Although chromogenes was not commonly found among the NAS isolated from IMI in this Brazilian study, genes related to production of enterotoxins were found in 66% (n = 85) of all CNS and in 35% of the CPS (SA) isolates.
    - *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.
  + ***Why we care about resistance and virulence in NAS***
    - In addition to their enterotoxigenic ability, staphylococci are able to develop resistance to various antimicrobials through different genetic mechanisms
      * Takahashi, 1998: *Characterization of gyrA, gyrB, grlA and grlB mutations in fluoroquinolone-resistant clinical isolates of Staphylococcus aureus*
      * Speer 1992: *Bacterial resistance to tetracycline: Mechanisms, transfer, and clinical significance*
    - Food-originated staphylococci carrying SCCmec and multiple antimicrobial resistance genes in the food production chain could be a substantial reservoir for transmission of the resistance genes *(Although these are all from meat- find dairy-specific ones?)*
      * Martins 2013: Coagulase-positive staphylococci isolated from chicken meat: Pathogenic potential and vancomycin resistance
      * Bhargava 2014 Characterization of methicillin-resistant coagulase-negative staphylococci (MRCoNS)in retail meat
      * Yurdakul 2013: Antibiotic resistance of enterococci, coagulase negative staphylococci and Staphylococcus aureus isolated from chicken meat
      * Osman, 2016: Prevalence of the antibiotic resistance genes in coagulase-positive-and negative-staphylococcus in chicken meat retailed to consumers
      * *CoNS have been reported to be resistant to a wide range of antibacterial agents including β-lactams, aminoglycosides, and tetracycline. Their resistance could eventually pose a public health hazard (from El-Ashker 2020; dairy specific examples – although these are all smaller studies in developing countries? Also, should check for chromogenes specifically)*
        + Khazandi 2018: *Genomic characterization of coagulase-negative staphylococci including methicillin-resistant Staphylococcus sciuri causing bovine mastitis*
        + Frey 2013: *Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk*
        + Gindonis 2013: *Occurrence and characterization of methicillin-resistant staphylococci from bovine mastitis milk samples in Finland*
        + Li 2015: *Characterization of methicillin-resistant and-susceptible staphylococcal isolates from bovine milk in Northwestern China*
        + Igbinosa 2019: *Characterization of antibiotic-resistant and species diversity of staphylococci isolated from apparently healthy farm animals.*
        + Simões 2020: Phenotypic antimicrobial susceptibility of environmental bacteria from mastitic milk of pastured dairy cows of S. Miguel (Azores).
        + El-Ashker 2020 *Antimicrobial resistance pattern and virulence profile of S. aureus isolated from household cattle and buffalo with mastitis in Egypt*

Chromogenes had no MDR isolates, but did have blaZ pen resistance

Other NAS were identified as MDR

* + - * + ***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*

Li et al. 2015; Khazandi 2018

* + - * + CoNS tend to be more resistant to antibacterial agents than S. aureus and can easily develop multi-resistance, in study below

Taponen 2009: Coagulase-negative staphylococci as cause of bovine mastitis—Not so different from Staphylococcus aureus?.

* + - 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
      * Shen 2013: *Presence and dissemination of the multiresistance gene cfr in Gram-positive and Gram-negative bacteria*
      * Feßler, 2018: *Mobile macrolide resistance genes in staphylococci*
      * Feßler, 2018: *Small antimicrobial resistance plasmids in livestock-associated methicillin-resistant Staphylococcus aureus CC398.*
    - 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
      * 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*

***Summary of risk factors for different species: Different animal factors and mgmt. factors influence what NASM species predominate for a farm, animal***

* Farm-level
  + Seasonality; Dolder 2017, De Visscher 2017
  + Geography; Swedish studies have epidermidis to be dominant NAS (person waller 2011 nyman 2018), finland was simulans (taponen 2016) vs everywhere else chromogenes, distribution of NASM species varied over the 4 regions of Canada Condas 2017 all canada prevalence paper
  + Bedding material (xylosus and succinus from sawdust, Piessens 2011)
  + Facility type:
    - Condas 2017, Canada prevalence; Simulans, xylosus, cohnii, saprophyticus, capitis, arlettae IMI higher in tiestall barns; prevalence of epidermidis was lowest; Chromogenes and sciuri highest in bedded pack barns
    - Adkins 2022; some species were specific to BP, some to SBF
  + Misc mgmt. practices
    - devischer 2017 BTM;
      * herds with **udders clipped** had lower odds of yielding chromogenes, simulans, xylosus (species more relevant for udder health)
      * milking protocol:
        + Herds not using single dry cotton or paper towel for each cow more likely to have cohnii
        + Herds that flushed milking units/steamed them after cow with SCIMI milked less likely to have haemolyticus, simulans, cohnii
        + Always wearing gloves during milking decreased devriesei
      * Tap water used for drinking from public supply increased odds of simulans
    - Hogan et al 1987
      * CNS species distribution affected by type of germicide used for teat dipping
* Animal- level
  + Udder edema; Dolder 2017; risk factor for chromogenes
  + Early stage of lactation (xylosus early, warneri mid-late); Dolder 2017
    - ***Species distribution can vary with stage of lactation***
      * Condas et al 2017 (Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds)
    - In Flanders, Staph chromogenes predominant species both at freshening and throughout lactation in randomly selected heifers and older cows; followed by Staph sciuri and Staph cohnii at parturition (De Visscher 2016); then Staph simulans, Staph xylosus, Staph epidermidis, Staph haemolyticus during lactation (Piessens et al 2011, Supre et al 2011)
  + De Visscher 2016
    - Teat anatomy and hygiene
      * Quarters with an inverted teat end had higher odds of being infected with chromogenes, simulans, or xylosus as well as with chromogenes solely
      * Dirty teats: more likely cohnii, equorum, saprophyticus, sciuri
  + Parity
    - Staph chromogenes, Staph xylosus, Staph simulans more commonly found in heifers than third-parity and older cows
      * Thorberg et al., 2009; De Visscher et al., 2016; Condas et al., 2017a (Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds); Nyman et al., 2018
    - Also found this in De Visscher 2016: heifers more likely to have these three species, or chromogenes solely
    - Mork 2012 found chromogenes more in primiparous animals, epidermidis more in multiparous animals