

Guide to interpretation of bovine mastitis culture results

The following is an overview of the more commonly identified organisms identified using bacteriological culture methods and more advanced techniques such as Maldi-TOF MS and qPCR.

This is not an extensive list, but outlines the main characteristics of the more commonly identified microorganisms which are identified on milk culture results.

Acinetobacter spp. Most likely of limited clinical significance and thought to make up part of the normal microbiome of the teat apex.

Aerococcus viridans: See Strep-like-organisms

Bacillus species. Commonly identified on bovine mastitis cultures. *Bacillus cereus* has been described as a cause of clinical mastitis, but *Bacillus* species (such as *Bacillus mycoides/ Bacillus subtilis*) are known to colonise the teat apex and may be protective in preventing the introduction/ establishment of other pathogens, such as *Staphylococcus aureus*.(Isaac et al., 2023) They are also commonly identified as part of a mixed growth on contaminated cultures which have not been taken aseptically. In general they should be disregarded as a significant cause of bovine mastitis.

Candida species including *Diutina rugosa* (*syn. Candida rugosa*): Sometimes cultured in pure growth from bovine quarters. They are generally associated with previous antimicrobial treatment for other mastitis pathogens. They are not susceptible to conventional antimicrobial treatments and therefore antimicrobial treatment is not advised for quarters infected with yeast species, with culling of affected animals often being the most advisable course of action.

Citrobacter spp. Opportunistic pathogen, member of Enterobacteriaceae family. Likely to be of limited significance in bovine mastitis

Corynebacterium spp. Common commensals of teat canal(Braem et al., 2012) *Corynebacterium bovis* being the most widely reported member of the genus. Some authors suggesting that it may offer protection from other pathogens by inhibiting the growth of major intramammary infecting pathogens (IMIs) (Woodward et al., 1985)Often associated with teat end damage or poor post milking teat disinfection. Can lead to subclinical mastitis / elevated somatic cell count (Gonçalves et al., 2016)

Diutina Rugosa ; See *Candida spp*

Enterobacter spp. Gram negative environmental pathogens. Maybe an environmental cause of clinical mastitis or subclinical mastitis. Usually associated with mild clinical signs and spontaneous cure is common. (Schukken et al., 2012)May also be contaminants where milk samples have not been taken aseptically.

Hafnia alvei: Formerly known as *Enterobacter alvei*. Member of the Enterobacteriaceae family of bacteria. Frequently isolated from milk samples. Maybe associated with chronic infections/ elevated somatic cell count (Padilla et al., 2015)

Kluyvera spp. : Significance unknown

Pantoea agglomerans: Formerly known as *Enterobacter agglomerans*. Similar characteristics to other *Enterobacter* spp.

Enterococcus spp. : See Strep-Like-Organisms

Escherichia coli: Common IMI in cows, causing clinical and subclinical mastitis. Clinical signs associated with the release of Lipopolysaccharide (LPS) from the cell wall. Classically *E.coli* mastitis has been associated with severe clinical signs within 1-2 weeks of calving. It has recently been suggested that some strains of Mammary Pathogenic *E.coli* (MPEC) have virulence factors which allow them to persist and cause chronic infections in the udder. These virulence factors include reduced stimulation of host immune response, biofilm production and the ability to invade mammary epithelial cells. In common with other members of the Enterobacteriaceae, infections are often acquired during the dry period, remaining dormant until they recrudescence to cause clinical disease within the first 100 days of calving. (Bradley et al., 2015)

Hafnia alvei : See *Enterobacter* spp

Klebsiella species. Environmental cause of bovine mastitis. Often associated with contaminated bedding, particularly sawdust used to dress cubicle beds. Commonly cultured from bovine faeces, and this is considered to be a more important source of infection than the introduction of fresh bedding on farms (Zadoks et al., 2011) Can cause severe clinical mastitis, but clinical signs not as severe as those associated with coliform mastitis. Duration of infection can be prolonged, with an associated protracted period of reduced milk production. (Schukken et al., 2012)

IMIs with this organism are usually associated with individual environmental exposure often due to faecal contamination of the teat ends, but outbreaks have been associated with contamination of rubber teat liners and the possibility of spread during the milking process. Response to treatment is often poor, with third generation cephalosporins showing the best response to treatment. (Schukken et al., 2012) Often present as a contaminant where samples have not been taken aseptically.

Raoultella spp.: *Raoultella* spp. are indistinguishable from *Klebsiella* spp using routine bacteriological methods. The genus was introduced following a re-classification of the *Klebsiella* genus. There are two species identified in bovine milk samples, *R.terrigena* and *R.planticola*. *R.planticola* is associated with soil and feed crops. (Schukken et al., 2012)

Lactococcus species: See Strep-Like-Organisms

Macrocooccus spp.: Closely related to *Staphylococcus* spp, have been rare reports of *Macrocooccus caseolyticus* associated bovine mastitis. The organism has been identified in bulk tank milk samples from England and Wales, with all of the isolates in that study showing methicillin resistance (MacFadyen et al., 2018). Little is known about the significance of *Macrocooccus* spp. as a mastitis pathogen, however they could pose a threat for the spread of methicillin resistance to *Staphylococcus aureus* bacterial populations on farms (MacFadyen et al., 2018) and therefore carriers should be regarded as posing a significant risk to the remainder of the herd.

Mycoplasma spp. Cause a highly contagious form of mastitis, usually affecting more than one quarter and causing a significant drop in milk production, with purulent/ abnormal secretions. Infected cows can remain visibly normal. (Nicholas et al., 2016) Diagnosis is usually by PCR testing on milk samples, the organism is not readily identifiable using routine culture conditions.

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Steps to manage Mycoplasma on farms

- PCR test bulk tank milk weekly to rapidly identify if infected cows are entering the tank. Carry out batch testing to identify individually infected cows if the tank turns positive on PCR
- Clean and disinfect milking equipment between milking sessions.
- Test milk samples from all cows before they enter or re-enter the lactating herd;
- Segregate cows found to be mycoplasma positive or showing mycoplasma mastitis to isolation pens and carefully monitor contacts.
- Cull cows where welfare is compromised.
- Antibiotic treatment should be discouraged when *M. bovis* has been confirmed.
- Once infected, cows should remain segregated for life unless milk is shown to be *M. bovis*-free over three successive monthly phases of testing
- Where *M. bovis* is suspected or confirmed, waste milk should be discarded or pasteurised before feeding to calves

(Nicholas et al., 2016)

Pantoea agglomerans : See Enterobacter spp.

Pasteurellaceae: *Mannheimia haemolytica* and *Pasteurella multocida* have been described as rare causes of bovine mastitis (Quinn et al., 2011)

Prototheca spp. Algae associated with wet areas containing manure and plant matter. When these organisms are identified on milk cultures, they are often associated with reduced milk production and elevated SCC. (Shahid et al., 2020) There is no treatment for *Prototheca* mastitis, with infections remaining chronic. Spontaneous recovery is not thought to happen and therefore cases should be culled to prevent spread to other cows (Suvajđić et al., 2017)

Pseudomonas spp.: Gram negative environmental pathogens. Often associated with contaminated water sources. Can cause severe clinical mastitis. (Biggs, 2009) Many different species identified eg *Pseudomonas aeruginosa*, *Pseudomonas koreensis*. *Pseudomonas spp.* are resistant to most commonly used antimicrobials. Often present as a contaminant where samples have not been taken aseptically.

Raoultella spp.: see *Klebsiella spp*

Serratia spp.: Environmental bacteria which may cause clinical or subclinical mastitis. Clinical episodes can alternate with subclinical infection. The duration of infection tends to be longer than that of other gram-negative organisms, with periods of between 55 days and 4 months being quoted, with some infections persisting for up to 3 years. (Schukken et al., 2012) Most cases are associated with *S. marcescens* or *S. liquefaciens*. Outbreaks have usually been associated with a single point source of contaminated chlorhexidine-based teat dip (contaminated on farm post opening). Clinical cases are usually mild, but infection is associated with dramatic increases in somatic cell count. Response to treatment is generally poor, with some authors reporting successful outcomes from the use of neomycin-containing intramammary infusions. The majority of cases resolve spontaneously. (Schukken et al., 2012)

Staphylococcus aureus. Major cause of contagious mastitis outbreaks on farms. Approximately 50% of isolates are resistant to penicillin (Department of Agriculture Food and the Marine, 2019). *S.aureus* infects the mammary gland tissue and establishes intracellular infections in the inflammatory cells including macrophages where it prevents the autophagocytic activity of the cell, thereby allowing persistent infections to become established (Cai et al., 2020). Additionally *S.aureus* produces biofilm which impairs the effectiveness of antimicrobials used to treat IMIs. (Cheng & Han, 2020). Chronic infections result in fibrosis and microabscessation of the udder, further impeding successive treatment attempts (Biggs, 2009)

Staphylococcus spp. excluding Staphylococcus aureus (Non-aureus Staphylococci/ NAS)

The NAS group of bacteria are made up of over 50 individual species, more than 10 of which can be found in bovine milk. (Valckenier et al., 2020) Individual species within the NAS group behave differently, with some displaying more contagious patterns of spread and others being more opportunistic or environmental-type pathogens. Some members of this group have been shown to be highly host-adapted with the ability to persist in the bovine udder and spread from cow to cow at milking, a good example of a host-adapted NAS species is *Staph chromogenes*, which is one of the more common species of NAS identified in bovine milk samples. (de Visscher et al., 2016) On the other hand, some species, such as *Staphylococcus haemolyticus* are generally regarded as opportunistic environmental pathogens, which can persist throughout the dry period and lead to an increased risk of clinical mastitis in the subsequent lactation (Rowe et al., 2021)

The significance of many of these NAS organisms for udder health is not fully understood, however recent publications have demonstrated the following:

- NAS are highly prevalent in first lactation cows. Heifers are more prone to have Intramammary infections with NAS either before or at the start of their first lactation with up to 45% of heifer quarters being infected at first calving. (Valckenier et al., 2020)
- Presence of NAS organisms in the udder is generally associated with elevated SCC, which in the case of some NAS organisms can persist into the subsequent lactation (eg *Staphylococcus chromogenes*). NAS organisms are not generally associated with clinical mastitis, but some authors have demonstrated an association between the presence of NAS organisms at drying off and an increased risk of clinical mastitis in the subsequent lactation (eg *Staph haemolyticus*, *Staph xylosus*) (Rowe et al., 2021)
- Elevated SCC in heifers associated with the presence of NAS in the udder is not necessarily associated with reduced milk production during the first lactation, with some authors demonstrating a slightly higher milk yield for NAS infected first lactation cows vs. those where a major mastitis organism (or no mastitis organisms) was identified. (Valckenier et al., 2020) Antimicrobial treatment of heifers with intramammary infections caused by NAS is generally not recommended and such infections should be differentiated from those caused by major mastitis pathogens (eg *S.aureus*, *S.uberis*) which do have a serious negative impact on the future productivity of the cow. (Piepers et al., 2010)
- The role played by NAS organisms in the udder is not clearly understood with some being shown to inhibit the growth of major mastitis pathogens such as *Staphylococcus aureus*, *Strep. uberis* and *Strep. dysgalactiae* (De Vlieghe et al., 2004). This suggests that some NAS organisms may be beneficial in preventing the growth of major mastitis pathogens in the udder, and therefore may act in a protective way as part of the natural microbiome of the udder.

Strep-Like-Organisms (SLO)

- Streptococcus like organisms are often identified in cows with elevated SCC, with the main organisms identified being *Aerococcus viridans*, *Lactococcus lactis*, *Lactococcus garvieae*, *Enterococcus faecalis*, *Enterococcus faecium*.
 - ***Aerococcus viridans***: Constitute part of the normal microflora of human skin and only cause issues in immunocompromised individuals (Braem et al., 2012). It is a common isolate in bovine milk samples from cows with high cell count at drying off. (Rowe et al., 2021) It is mainly detected in mixed cultures of milk from cows with subclinical mastitis and may form part of the normal microbiome of the teat apex. (Braem et al., 2012) Little is known about its significance as a mastitis pathogen, but it has been shown to be associated with reduced milk yield. (Sun et al., 2017) This may be due to its common co-infection with other more significant pathogens. Some authors suggest that it has little significance as a mastitis pathogen (Wyder et al., 2011) and may actually offer protection against infection (Woodward et al., 1985.)The presence of *Aerococcus* at drying off is not associated with increased risk of clinical or subclinical mastitis in the subsequent lactation and therefore does not warrant antimicrobial treatment. (Rowe et al., 2021)
 - ***Enterococcus spp.*** Gram positive environmental pathogens associated with bedding They produce biofilms which make them difficult to treat (Elhadidy & Zahran, 2014) and are associated with persistent infections across the dry period leading to subclinical and clinical mastitis in the subsequent lactation (Rowe et al., 2021) They can be resistant to a range of antimicrobials and are intrinsically resistant to cephalosporins and isoxazolyl penicillins such as cloxacillin (Sorge et al., 2021).
 - ***Lactococcus spp.*** Gram positive environmental pathogens, the two species reported in bovine milk samples are *Lactococcus lactis* and *Lactococcus garvieae*. Associated with elevated somatic cell count, not generally associated with clinical mastitis, but outbreaks of *Lactococcus* associated clinical mastitis have been reported (Rodrigues et al., 2016) *Lactococcus garvieae* are intrinsically resistant to isoxazolyl penicillins such as cloxacillin (Sorge et al., 2021).

Streptococcus dysgalactiae. Generally associated with poor teat condition and blackspot. Usually responds well to treatment. Some regard it as an opportunistic environmental pathogen. Unlikely to be a cause of high BTM SCC. (Biggs 2009)

Streptococcus uberis. Opportunistic environmental pathogen, over 500 strains exist. Some are more host-adapted (contagious) than others. Sensitive to treatment with penicillin. Reports of treatment failures with penicillin more likely to be *Enterococci spp.* which have been incorrectly identified. (Sherwin & Breen, 2022)

Trueperella pyogenes : Often associated with “summer mastitis”. Spread by flies. Results in production of purulent material and usually the loss of the infected quarter. (Rzewuska et al., 2019)

Many other bacteria are identified on bovine milk samples. In all cases there is a clinical judgement to be made as to whether the organism identified is significant at the cow or herd level as a cause of mastitis.

Conclusions

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The increased use of advanced diagnostic testing methods has allowed the identification of a broader range of intramammary infections than was previously possible. This allows greater understanding of the aetiological agents contributing to bovine mastitis on farms. In all cases the practitioner should assess the likely contribution of the microorganisms identified to the overall mastitis situation on the farm. Where repeated growths of pure culture of individual bacterial species are shown on multiple milk samples it is more likely that they are clinically or sub clinically significant.

Many of the less common bacteria may not be significant contributors to clinical mastitis or elevated BTM SCC levels. In some cases these bacteria are more likely to be contaminants in the milk sample due to poor sampling technique and should not be regarded as significant for mastitis control on the farm, in which case the antimicrobial sensitivity results for these bacteria will not be relevant when selecting mastitis treatments at herd level.

The common identification of organisms with intrinsic resistance to antimicrobials such as *Enterobacter spp.* and Non-aureus staphylococci highlights the need for veterinary practitioners to be more selective in the treatments used for mastitis cases and indeed whether a decision should be made not to treat mastitis cases with bacteriological aetiologies which are deemed to be relatively benign such as the non-aureus staphylococci, or which pose a risk to the spread of antimicrobial resistance within the herd, such as *Enterococci spp.* or *Macrocococuss spp.*

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