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# *Invited review:* Antimicrobial resistance in bovine mastitis pathogens: A review of genetic determinants and prevalence of resistance in European countries

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## ABSTRACT

Antimicrobial resistance is an urgent and growing problem worldwide, both for human and animal health. In the animal health sector actions have been taken as concerns grow regarding the development and spread of antimicrobial resistance. Mastitis is the most common infection in dairy cattle. We aimed to summarize the genetic determinants found in staphylococci, streptococci, and *Enterobacteriaceae* isolated from mastitic milk samples and provide a comparison of percentage resistance to a variety of antimicrobials in European countries.
**Key words:** AMR, pathogens, mastitis, resistance genes

## INTRODUCTION

Bovine mastitis causes an economic loss to the dairy industry worldwide. In Ireland, it has been estimated that a 30% decrease in bulk milk somatic cell count levels could increase industry returns by €37.7 million per annum (Geary et al., 2013). Control of mastitis can be challenging, due to the high number of subclinical infections that go undetected but still lead to reduced milk production, and to the withdrawal periods that treatment in dairy cattle follow to control for antimicrobial residues in milk for human consumption. In addition, the increase of resistance worldwide has demonstrated how identification of the causing agents and their resistance patterns is key for appropriate antimicrobial therapy of infected animals (Ruegg, 2021).

Antimicrobial resistance (**AMR**) is a rising threat, with new and emerging mechanisms of resistance appearing and spreading globally. As antibiotics become less effective, certain infections are becoming harder, and even impossible, to treat. Public health concerns are, therefore, increasing with the growing challenge that AMR poses. The presence of pathogenic bacteria in milk is considered a Food Safety issue through the consumption of raw milk (https://www.cdc.gov/foodsafety/rawmilk/raw-milk-questions-and-answers.html), while living or working in close contact with dairy cattle increases infection risk (Juhász-Kaszanyitzky et al., 2007). Methicillin-resistant *Staphylococcus aureus* (**MRSA**) and multidrug resistant gram-negative bacteria have been detected in raw milk or raw-milk products (Skočková et al., 2015; Herrera et al., 2016). Therefore, surveillance of zoonotic pathogens in animals is one of the European Union's (**EU**) goals. However, nonzoonotic pathogens are also a threat, first to animal health and welfare, but also to public health as they are a source of transferable genetic resistance.

The magnitude of the AMR problem has resulted in it being a high priority for health policy makers worldwide, with many implications that will affect the human health, animal health, and environmental sector (One Health) in the future. However, surveillance of veterinary clinical isolates is not systematically performed and can be challenging, as there are gaps in the knowledge and tools needed to correctly evaluate the bacteria (Mader et al., 2022).

The aim of this review is to summarize the current situation of AMR identified in the main pathogens causing bovine mastitis. Genetic determinants detected worldwide in *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, and *Klebsiella pneumoniae* are discussed. Additionally, prevalence of resistance to different antimicrobials and the future of surveillance programs are exposed with a focus in Europe.

## ETIOLOGY OF MASTITIS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

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Mastitis is inflammation of the mammary gland resulting as a consequence of microbial infection (Blowey and Edmondson, 2010). Many bacterial species, yeasts, or fungi have been isolated from the mammary gland (Watts, 1988). However, the etiological agents most commonly found to be involved in bovine mastitis are *Staph. aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *E. coli*, CNS, and other *Streptococcus* spp. (such as *Streptococcus dysgalactiae*; Keane et al., 2013; Poutrel et al., 2018; Krishnamoorthy et al., 2021). These microorganisms can roughly be classified as contagious or environmental pathogens. The first group includes microorganisms adapted to survive within the mammary gland (such as *Staph. aureus* or *Strep. agalactiae*, for example) and their transmission is primarily from cow to cow, mostly during the milking process (Blowey and Edmondson, 2010). In contrast, environmental pathogens come from the contaminated environment, are opportunistic invaders, and their infections usually take place between milkings and during the dry period (for example, *Strep. uberis* or *E. coli* and other coliforms; Bradley, 2002).

Intramammary infections can occur in clinical, subclinical forms, or asymptomatic forms. Clinical forms may take an acute or chronic course of infection, but pathological signs such as swelling, heat, hardness, redness or pain of the udder, changes in the milk appearance, and reduction of milk yield can always be observed (Argaw, 2016). However, subclinical infections are not visible externally, but cause production losses and changes in milk parameters.

Botrel et al. (2010) established *Strep. uberis*, *E. coli*, and coagulase-positive staphylococci as the major causative agents of clinical mastitis in France, and CNS and *Strep. dysgalactiae* to be predominantly implicated in subclinical mastitis. The epidemiology of mastitis has changed in the last 70 years, due to the introduction of mastitis control strategies and regulations on milk and milk products. For these reasons, environmental pathogens are now a more common cause of mastitis than they were previously (Bradley, 2002).

## GENETIC DETERMINANTS OF RESISTANCE IN MASTITIS PATHOGENS: GRAM-POSITIVE MICROORGANISMS

As indicated above, several bacterial species can cause mastitis. This section summarizes the most common acquired AMR mechanisms employed by both gram-positive and gram-negative organisms isolated from bovine milk samples. Gram-positive microorganisms responsible for mastitis infections are mainly from the *Staphylococcus* and *Streptococcus* genera.

### *Staphylococcus*

*Staphylococcus aureus* is the main cause of clinical bovine mastitis. It is a common commensal of nares and skin, and can become an opportunistic pathogen leading to superficial and invasive infections both in humans and animals (Lowy, 1998; Foster and Geoghegan, 2015). It expresses a plethora of surface proteins, some of which are shared with CNS, that act as virulence factors with functions such as adhesion to surfaces, promotion of biofilm formation, invasion of epithelial cells, or immune evasion (Foster et al., 2014). In addition, CNS are frequently isolated from bovine milk, usually causing subclinical mastitis (Pyörälä and Taponen, 2009). Examples of these are *Staphylococcus haemolyticus*, *Staphylococcus xylosus*, *Staphylococcus hominis*, *Staphylococcus hyicus*, *Staphylococcus warneri*, *Staphylococcus sciuri*, *Staphylococcus simulans*, *Staphylococcus chromogenes*, and *Staphylococcus epidermidis*, among others (Li et al., 2015; Wendlandt et al., 2015a; Khazandi et al., 2018).

With regard to intrinsic or natural resistance, *Staph. aureus* showed reduced fitness in the presence of ciprofloxacin, daptomycin, gentamicin, linezolid, oxacillin, or vancomycin during the activation of intrinsic factors such as mprF, ndh, fmtA, graR, or dltA (Blake and O'Neill, 2013; Rajagopal et al., 2016). Activation of some chromosomically encoded multidrug-efflux pumps such as NorA or LmrS can confer resistance or reduced susceptibility to quinolones or lincomycin, respectively (Floyd et al., 2010; Costa et al., 2013). In addition, novobiocin is a coumarin antibiotic to which CNS, such as *Staphylococcus saprophyticus*, *Staph. sciuri*, *Staphylococcus vitulinus*, *Staphylococcus fleuretti*, *Staphylococcus cohnii*, *Staphylococcus equorum*, *Staphylococcus kloosii*, *Staphylococcus arlettae*, *Staphylococcus gallinarum*, *Staphylococcus nepalensis*, *Staphylococcus succinus*, or *Staph. xylosus*, are intrinsically resistant due to the expression of a novobiocin-resistant GyrB protein (Vickers et al., 2007; Nobrega et al., 2018b). Finally, a β-lactamase encoding gene, $bla_{\mathrm{ARL}}$, has been identified in *Staphylococcus arlettae* (CNS) from bovine mastitic milk in Switzerland and Canada, located in the chromosomal DNA with its regulatory genes $blaI_{\mathrm{ARL}}$ and $blaR1_{\mathrm{ARL}}$ (Andreis et al., 2017; Nobrega et al., 2018a).

Intrinsic resistance factors do not always confer full resistance, and may be presented as a lower phenotypic susceptibility to the particular antimicrobial. It is also possible to find acquired resistance mechanisms in isolates where intrinsic resistance factors are present. *Staphylococcus* spp. isolated from bovine mastitis cases have been reported to acquire resistance to several antimicrobial classes including β-lactams, tetracyclines,

aminoglycosides, amphenicols, macrolides, trimethoprim, lipopeptides, and lincosamides (Lowy, 2003; Pantosti et al., 2007; Wendlandt et al., 2013a; Nobrega et al., 2018b; Table 1).

***Resistance to β-Lactams.*** The increase in penicillin-resistant isolates encountered during the 1940s and 1950s led to the introduction of another β-lactam antimicrobial, methicillin, as a therapeutic solution. Methicillin is a semisynthetic penicillinase-resistant penicillin. However, shortly after methicillin-resistant MRSA was detected. Even though it was first reported in a British hospital in the early 1960s, MRSA is now a worldwide cause of healthcare, community, and livestock infections (Lyon and Skurray, 1987; Lowy, 2003).

The genetic basis for resistance against both β-lactam antimicrobials, penicillin, and methicillin differs in their genetic elements and location within the cell, but the regulation mechanisms have some similarities. The *bla*Z gene encodes the PC1 β-lactamase responsible for penicillin resistance both in *Staph. aureus* and CNS species, including isolates from mastitis such as *Staph. epidermidis*, *Staph. haemolyticus*, or *Staph. chromogenes* (Olsen et al., 2006; Nobrega et al., 2018a; Bolte et al., 2020b). The *bla*Z gene is located in the mobile genetic element transposon Tn552, either in the bacterial chromosome or a plasmid, and is under the control of 2 regulatory genes, *bla*R1 and *bla*I, organized in the blaZ-blaR1-blaI operon (Olsen et al., 2006; Llarrull and Mobashery, 2012).

Resistance to methicillin and oxacillin is mediated by the *mec*A gene, which encodes PBP2a (with low β-lactam affinity), and its expression is regulated by the inducer-repressor genes *mec*R1 and *mec*I (Blázquez et al., 2014). The *mec*A gene is located with its regulators (mec gene complex) and a cassette chromosome recombinase (ccr gene complex) responsible for the mobility of the system, in a mobile genetic element named staphylococcal cassette chromosome mec (**SCCmec**) which integrates in the bacterial chromosome always at the same site (att site within host chromosomal gene *orf*X; Katayama et al., 2000; Ito et al., 2009). The transfer mechanism is still not fully understood, but conjugation, transduction, and transformation have been reported (Cafini et al., 2017). The *bla* regulatory system can also regulate *mec*A, particularly when *mec*R1 and *mec*I are not present (Hackbarth and Chambers, 1993; Liu et al., 2016). Thirteen different types of SCCmec have been discovered to date and a *mec*A homolog, *mec*C, has also been found to confer resistance to penicillinase-resistant penicillins (Ballhausen et al., 2014; Baig et al., 2018). This was first isolated in England and Denmark from human and bovine isolates, but has also been detected elsewhere (García-Álvarez et al., 2011). In fact, herd-level prevalence of *mec*C isolates in English and Welsh dairy farms was 2.15% (Paterson et al., 2014). It has been shown that Africa, Latin America, and Asia have higher levels of oxacillin or cefoxitin resistance than Europe or North America (Molineri et al., 2021).

The CNS are thought to have a role in the spread of resistance within staphylococcal species isolated from clinical and other public and environmental settings, or animals. Indeed, CNS can carry *mec*A genes in SCCmec elements, show resistance to oxacillin, and ancestral forms of SSCmec have been identified in some species (Hussain et al., 2000; Cafini et al., 2017; Saber et al., 2017; Xu et al., 2018). The *mec*A gene homologs such as *mec*A1 or *mec*A2 have been identified in *Staph. sciuri* or *Staph. vitulinus*, respectively; however, these do not confer β-lactam resistance (Miragaia, 2018). Methicillin resistance due to *mec*A is commonly observed in *Staphylococcus* spp. causing bovine mastitis infections (Turutoglu et al., 2009; Soares et al., 2012; Pu et al., 2014; Khazandi et al., 2018). However, methicillin-susceptible isolates of *Staph. aureus* from bovine mastitis carrying the *mec*A gene have been detected; it is therefore important to combine genotypic and phenotypic tests to obtain certain results (Pu et al., 2014).

***Resistance to Tetracyclines.*** Resistance to tetracyclines in *staphylococcal* species from mastitis samples is mainly related to the genes *tet*(K) and *tet*(L), which code for membrane-associated efflux proteins of the Major Facilitator Superfamily, and are transferred by plasmids (Enany and Alexander, 2017; Schwarz et al., 2018). Gene *tet*(38) is a chromosomally encoded efflux pump that can be overexpressed from a plasmid (Truong-Bolduc et al., 2014; Chen and Hooper, 2018). Additionally, the gene *tet*(M) is also frequently found and codes for a ribosome-protective protein. The *tet*(M) gene is usually located in conjugative transposons Tn916-Tn1545 (Liu et al., 2017; Schwarz et al., 2018).

These genes were detected in *Staph. aureus* and CNS from dairy farms in Switzerland, Canada, China, Australia, or Germany, for instance (Feßler et al., 2010; Frey et al., 2013; Ali and Shrief, 2016; Khazandi et al., 2018; Nobrega et al., 2018b; Qu et al., 2019; Lima et al., 2020). Qu et al. (2019) reported that the genes *tet*(K), *tet*(L), *tet*(M) were more common in non-*aureus* isolates than in *Staph. aureus*. It is possible to find combinations of 2 or more genes in the same isolate, particularly *tet*(K) and *tet*(M) (Wendlandt et al., 2013a).

***Resistance to Aminoglycosides and Aminocyclitols.*** Resistance to aminoglycosides can be mediated through several genes that code for inactivating enzymes in staphylococcal species. The gene *aph*A3 codes for a phosphotransferase and mediates resistance to kanamycin, neomycin, and amikacin. In contrast, *aac*A-*aph*D codes for an acetyltransferase and phosphotransferase conferring resistance to gentamicin, kanamycin,

**Table 1.** Percent of resistance of *Staphylococcus* from bovine mastitis to various antimicrobials in EU countries[1]

| Country | T[2] | Disc[3] or MIC | Standard[4] | Pen | Amp | Amc | Oxa/Fox | Fur | Ery | Pir | Lin | Cli | Spc | Gen | Kan | Neo | Str | Cip | Enr | Tet | Smx | Tmp | Sxt | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | | | | | | | | | | | | | | | | | | | | | | | | |
| Austria (n = 100) | D | MIC | CLSI/ publications | 10 | — | — | 2 | — | 41* | 41 | — | — | — | — | — | — | — | — | — | — | — | — | — | Wald et al., 2019 |
| Belgium (n = 768) | D | Disc | CLSI | — | 12.7 | 2.1 | 2.6 | — | 3.1 | — | — | — | — | — | — | — | — | — | — | 8.3 | — | — | 1.8 | Supré et al., 2014 |
| Czech Republic (n = 46) | S | Disc | CLSI | 65 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Baumgartner et al., 2018 |
| Croatia* (n = 140) | D | Disc | CLSI | 51.4 | — | 8.6 | 60*** | — | — | — | 31.4 | — | — | — | — | — | — | — | 11.4 | 62.2** | — | — | 45.7 | Sukalić et al., 2021 |
| Denmark (n = 63) | D | MIC | CLSI/ EUCAST | 17.5 | — | — | 1.6 | — | 4.8 | — | — | — | 52.4 | 0 | — | — | 0 | 0 | — | 1.6 | 28.6 | 0 | 0 | Chehabi et al., 2019 |
| England and Wales (n = 28) | D | Disc | BSAC/ AHVLA | 17.9 | — | 7.1 | — | — | — | — | — | — | — | — | — | 0 | — | — | — | 7.1 | — | — | — | UK-VARSS, 2020 |
| Finland (n = 196) | S | MIC | NCCLS/ SVARM | 52.1 | — | — | 4.1 | — | 1.5 | — | — | 0.5 | — | 0 | — | 0 | 4.1 | — | 0 | 5.1 | — | — | 1.5 | Pitkälä et al., 2004 |
| France * (CPS) (n = 401) | D | Disc | CA-SFM | 17 | — | — | 8 | — | 5 | — | 2 | — | — | 1 | 1 | 2 | 13 | — | 0 | 4 | — | — | 2 | Anses, 2021 |
| Germany (n = 56) | S | Disc | EUCAST | 85.7 | — | — | 0 | — | 0 | — | — | — | — | 10.7 | — | — | — | — | — | 7.1 | — | — | — | El Behiry et al., 2012 |
| Italy (n = 120) | D | Disc | CLSI/ CASFM | 64.5 | — | 9.4 | 13.1 | — | 42.9 | — | 86.5 | — | — | — | — | — | — | — | 17.5 | 25.1 | — | — | 3.4 | Intorre et al., 2013 |
| Ireland (n = 154) | D | Disc | CLSI | 44.8 | — | — | 0 | 0 | 3.2 | 1.9 | — | — | — | — | — | — | — | — | — | 1.9 | — | — | 0 | DAFM et al., 2021 |
| Norway (n = 20) | D | Disc | EUCAST | — | 5 | 0 | — | — | 0 | — | — | 0 | — | 5 | — | — | — | 0 | | 0 | — | 5 | 0 | Fergestad et al., 2021 |
| Lithuania (n = 176) | S | Disc | CLSI | 76.7 | 78.4 | 38.1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Klimiene et al., 2011 |
| Portugal (n = 28,126) | D | Disc | CLSI | 44.7 | — | 12.3 | 2.9 | — | — | — | — | — | — | 2.2 | — | — | — | — | 0 | — | — | — | 0.7 | Rocha et al., 2014 |
| Slovakia (n = 65) | S | Disc | CLSI/ EUCAST | 10.8 | — | — | 6.1† | — | — | — | 10.8 | — | — | — | — | 21.5 | 44.6 | — | — | 10.8 | — | — | — | Holko et al., 2019 |
| Sweden (n = 227) | S | MIC | EUCAST | 2.6 | — | — | 0.6 | — | 0 | — | — | 0.9 | — | 0 | 0.8 | — | — | 0 | — | 0.9 | — | 8.8 | 0 | Duse et al., 2021 |
| Switzerland (n = 58) | S | MIC | CLSI | 14 | — | — | 2 | — | 0 | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | Käppeli et al., 2019 |
| Ukraine (n = 59) | D | Disc | CLSI | — | — | — | — | 41.51 | — | — | — | — | 16.95 | — | — | — | — | — | — | 21.43 | — | — | — | Elias et al., 2020 |
| CNS | | | | | | | | | | | | | | | | | | | | | | | | |
| Austria (n = 100) | D | MIC | CLSI/ publications | 17 | — | — | — | — | 42 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Wald et al., 2019 |
| Czech Republic (n = 68) | S | Disc | CLSI | 43 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Baumgartner et al., 2018 |
| Croatia* (n = 189) | D | Disc | CLSI | 52.1 | — | 16.1 | 64.1*** | — | — | — | 50.8 | — | — | — | — | — | — | — | 18 | 79.9** | — | — | 53.4 | Sukalić et al., 2021 |
| Denmark (n = 49) | D | MIC | CLSI/ EUCAST | 22.4 | — | — | 2 | — | 0 | — | — | — | 10.2 | 2 | — | — | 0 | 0 | | 10.2 | 20.4 | 8.2 | 0 | Chehabi et al., 2019 |
| Finland (n = 400) | S/D | MIC | EUCAST | 41.8 | — | — | 34 | — | 6.3 | — | — | — | — | 0.8 | 2.3 | — | 7.5 | 0 | 0 | 16.5 | — | 50 | 5.3 | Taponen et al., 2016 |
| France* (n = 488) | D | Disc | CASFM | 26 | — | — | 3 | — | 12 | 8 | 21 | — | — | 1 | 2 | 3 | 19 | — | 1 | 18 | — | — | 3 | Anses, 2019 |

*Continued*

**Table 1 (Continued).** Percent of resistance of *Staphylococcus* from bovine mastitis to various antimicrobials in EU countries[1]

| Country | T[2] | Disc[3] or MIC | Standard[4] | Pen | Amp | Amc | Oxa/Fox | Fur | Ery | Pir | Lin | Cli | Spc | Gen | Kan | Neo | Str | Cip | Enr | Tet | Smx | Tmp | Sxt | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Germany (n = 14) | S | Disc | CLSI/ EUCAST | 74.28 | — | — | 0 | — | 0 | — | — | — | — | 10 | — | — | — | — | — | 7.14 | — | — | — | Behiry et al., 2012 |
| Lithuania (n = 95) | S | MIC | EUCAST | 67.4 | — | — | 4.2 | — | 13.7 | — | — | — | — | 9.5 | — | — | — | 4.2 | — | 18.9 | — | — | 10.5 | Klimiene et al., 2016 |
| Portugal (n = 204) | S | Disc | CLSI | — | — | — | 9.3 | — | — | — | — | — | — | — | — | — | — | 0 | 0 | — | — | — | 16.7 | Seixas et al., 2014 |
| Slovakia (n = 187) | S | Disc | CLSI/ EUCAST | 5.9 | — | — | 14.4† | — | — | — | 4.8 | — | — | — | — | 20.9 | 36.4 | — | — | — | — | — | — | Holko et al., 2019 |
| Sweden (n = 21) | S | MIC | EUCAST | 30.4 | — | — | 6.2 | — | 9.5 | — | — | 14.3 | — | 0 | — | — | — | 0 | — | 0 | — | — | — | Duse et al., 2021 |

[1]Pen: penicillin; Amp: ampicillin, Amc: amoxicillin + clavulanic acid; Oxa: oxacillin; Fox: cefoxitin; Fur: ceftiofur; Ery: Erythromycin; Pir: pirlimycin; Lin: lincomycin; Cli: clindamycin; Spc: spectinomycin; Gen: gentamicin; Kan: kanamycin; Neo: neomycin; Str: streptomycin; Cip: ciprofloxacin; Enr: enrofloxacin; Tet: tetracycline; Smx: sulfamethoxazole; Tmp: trimethoprim; Sxt: sulfamethoxazole + trimethoprim. In the case of *Staph. aureus* from Portugal, cloxacillin was tested instead of Oxa/Fox.

[2]T = type: D, diagnostic; S, survey. CPS: coagulase-positive staphylococcus.

[3]Disc = disc diffusion.

[4]CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; CA-SFM: Comité de l'Antibiogramme–Société Française de Microbiologie; BSAC: British Society for Antimicrobial Chemotherapy; AHVLA: Animal Health and Veterinary Laboratories Agency.

*Percent resistance was calculated using the % susceptibility published.

**Oxytetracycline was tested.

***Oxacillin was tested.

†Cloxacillin was tested.

tobramycin, and amikacin if overexpressed (Schwarz et al., 2018). Both can be localized in a transposon, on a plasmid, or in the bacterial chromosome. In a recent study about staphylococci from bovine mastitis in China, *aph*A3 was detected at a higher proportion in non-*aureus* than *Staph. aureus* isolates, whereas *aac*A-*aph*D was more commonly found in *Staph. aureus* (Qu et al., 2019). Both genes have also been identified in MRSA from Germany or Turkey, increasing the resistance spectrum of these isolates (Turutoglu et al., 2009; Feßler et al., 2010). The gene *aad*D (kanamycin, neomycin, and tobramycin resistance) has been identified both in *Staph. aureus* and non-*aureus* isolates from cases of bovine mastitis (Feßler et al., 2010; Qu et al., 2019). In addition, the genes *aad*E, *ant*(6)-Ia, and *str* mediate streptomycin resistance, and together with the genes *lsa*(E) and *lnu*(B), *aad*E is part of a multiresistant gene cluster. They have been found in mastitis *Staph. aureus* and CNS isolates (Frey et al., 2013; Silva et al., 2014; Wendlandt et al., 2015a; Antók et al., 2020). *Staphylococcus aureus* mastitis isolates from Colombia showed the presence of aminoglycoside resistance genes *aph*(3′)IIIa (kanamycin, neomycin, amikacin, gentamicin B, paromomycin), *ant*(4′)Ia3 (tobramycin, amikacin), or *aac*(6′)/*aph*(2″)-3 (gentamicin, tobramycin, amikacin), whereas in China the latest was found along with *aph*(3′)-III (kanamycin, neomycin, amikacin, gentamicin B, paromomycin; Wang et al., 2015; Jiménez Velásquez et al., 2020). In addition, Frey et al. (2013) showed that CNS from bovine mastitis milk from Switzerland also carry the aminoglycoside resistance genes *ant*(6)-Ia (streptomycin), *aac*(6′)-Ie–*aph*(2′)-Ia (gentamicin, tobramycin, amikacin), or *aph*(3′)-III (kanamycin, neomycin, amikacin, gentamicin B, paromomycin).

In the case of resistance to aminocyclitols, genes *spc* and *spw* (spectinomycin resistance genes) have been detected in methicillin-resistant *Staph. aureus* and CNS from bovine mastitis (Feßler et al., 2010; Frey et al., 2013; Silva et al., 2014; Wendlandt et al., 2015a; Kadlec et al., 2019).

Recent studies from China have detected class 1 integrons and gene cassettes in *Staph. aureus* from bovine mastitis (Li and Zhao, 2018). Gene cassettes *dfr*A1-*aad*A1, *aad*A2, *dfr*A12-*orf*X2-*aad*A2, and *aad*A1 were most prevalent in China, and all isolates were phenotypically resistant to aminoglycosides, with some also showing trimethoprim-sulfamethoxazole resistance (Li and Zhao, 2018). Abd El-Rahman et al. (2021) identified a class 1 integron in *Staph. aureus* of ruminant origin containing a *dfr*A15 gene cassette. These are considered a new finding, as integrons in gram-positive bacteria are not well characterized. However, the number of studies showing similar findings from human isolates as well is increasing, resulting in multidrug resistance in *Staph. aureus* associated with the presence of class 1 or class 2 integrons (Mostafa et al., 2015).

***Resistance to Macrolides or Lincosamides or Streptogramins.*** Macrolide, lincosamide, and streptogramin B resistance may be associated. This may be caused by certain genes that confer resistance to antimicrobial classes that share the same mode of action. In this case, expression of one or more *erm* class A, B, C, T, 43, 44, or 48 genes can induce a modification of the target site at the rRNA and inhibit the binding of these compounds to the ribosome (Wipf et al., 2014; Schwarz et al., 2018). Common erm genes reported from bovine mastitis MRSA in German studies include *erm*(A), *erm*(B), *erm*(C), and *erm*(T) (Feßler et al., 2010; Kadlec et al., 2019). The *erm*(A) and *erm*(B) genes are associated with transposons Tn554 and Tn917/Tn551. The *erm*(A) gene is usually integrated into SCCmec elements and *erm*(B) is related to multiresistant plasmids (Wendlandt et al., 2015b; Schwarz et al., 2018). The *erm*(C) gene is usually located in small plasmid that does not carry further resistant genes (Lodder et al., 1997). The *erm*(B) and *erm*(C) genes were found in CNS and *Staph. aureus* from mastitis cases in China (Li et al., 2015). The *erm*T gene can be harbored in a multiresistant plasmid and has been found to be more common in *Staph. aureus* than non-*aureus* staphylococci of bovine mastitis origin (Qu et al., 2019).

Other genes that employ different mechanisms are *msr*, *mph*, *ere*, *Inu*, vga, *lsa*, or *sal* genes. *msr*(A) and *msr*(B), which code for an efflux pump from the ABC-F subfamily protein, confer resistance to macrolide and streptogramin B. *mph*(C) codes for a macrolide phosphotransferase that inactivates some macrolide antibiotics, and *ere*(A) codes for an esterase that hydrolyzes the macrocyclic nucleus. *lnu*(A) and *lnu*(B) encode nucleotidyltrasferases and confer resistance to lincosamides (Schwarz et al., 2018). Resistance to lincosamides can also be achieved by the expression of plasmid genes *vga*(A), *vga*(C), *lsa*(E) and *sal*(A), which code for ABC transporters and mediate resistance to pleuromutilins, streptogramin A and lincosamides (Gentry et al., 2008; Kadlec et al., 2010; Hot et al., 2014; Wendlandt et al., 2015a, 2013b). Most of these genes have been detected both in *Staph. aureus* and CNS from mastitis origin (Lüthje et al., 2007; Feßler et al., 2010; Silva et al., 2014; Li et al., 2015; Liu et al., 2017; Nobrega et al., 2018b; Qu et al., 2019; Antók et al., 2020). However, *sal*(A) has only been detected in *Staph. sciuri* (CNS) from bovine mastitis to date (Wendlandt et al., 2015a).

***Other Resistance Genes.*** Other resistance genes that have been detected in staphylococci from bovine mastitis include those conferring resistance to

trimethoprim [*dfr*(A), *dfr*(D), *dfr*(G), *dfr*(K)], fluoroquinolones (*gyr*A mutation, *grl*A mutation, *mep*A), phenicols (*fex*A), vancomycin (*van*A), or sulfonamides (AMR-associated residues in the *fol*P gene; Feßler et al., 2010; Frey et al., 2013; Silva et al., 2014; Wendlandt et al., 2015a; Nobrega et al., 2018b; Qu et al., 2019; Antók et al., 2020; Naushad et al., 2020; Pérez et al., 2020; Ndahetuye et al., 2021; Patel et al., 2021). Some staphylococci not intrinsically resistant to novobiocin including *Staph. aureus* can acquire this resistance by accumulation of point mutations in the genes *par*E and *gyr*B (Fujimoto-Nakamura et al., 2005).

### Streptococcus

The *Streptococcus* spp. most commonly isolated from bovine mastitis samples in Ireland are *Strep. uberis* and *Strep. dysgalactiae* (DAFM et al., 2021). This was also true in studies from France, Sweden and Finland, but not in Portugal or Germany where *Strep. agalactiae* had a similar or higher prevalence than *Strep. uberis* and *Strep. dysgalactiae* (Rato et al., 2013; Vakkamäki et al., 2017; Poutrel et al., 2018; Bolte et al., 2020a; Duse et al., 2021).

Streptococci typically have low-level intrinsically resistant to quinolones due to the overexpression of ABC efflux pumps PmrA, PatA, or PatB, for instance (Garvey and Piddock, 2008). Low-level intrinsic resistance to aminoglycosides can also be present in streptococci as they are facultative anaerobic bacterium, whereas they are fully resistant to fusidic acid (Leclercq et al., 2013; El Moujaber et al., 2017). Acquired resistance from mastitis streptococci isolates is described below (Table 2).

***Resistance to Macrolides or Lincosamides or Streptogramins.*** Macrolide, lincosamide, and streptogramin B resistance phenotypes are observed in streptococci from bovine mastitis. Rato et al. (2013) identified the gene *erm*(B) in *Strep. uberis*, the genes *erm*(A) or *erm*(B) in *Strep. agalactiae*, and both *erm*(A) and *erm*(B) in *Strep. dysgalactiae* isolates. The mechanism of resistance employed by the expression of these genes is target protection, following methylation of the ribosome at position 2058 (Haenni et al., 2018). They are usually found in mobile genetic elements and can be associated with tetracycline resistance.

The *erm*B are among the most commonly detected genes that result in a macrolide, lincosamide, and streptogramin B resistant phenotype, but others such as *mef*, *msr*, or *mre* families, which code for efflux pumps, are also significant (Haenni et al., 2018, 2011). In a study from the United States, only *erm*(B) was present in *Strep. uberis* (Loch et al., 2005). In Germany *erm*(B) was only detected in *Strep. uberis*, but *erm*(B) and *erm*(C) were detected in *Strep. agalactiae* and *Strep. dysgalactiae*. Additionally, *mef*(A) and *msr*(D) were present in all 3 species (Entorf et al., 2016). Combination of these genes in the same isolate is very common. Duarte et al. (2005) found the *mre*A gene in 9/38 *Strep. agalactiae* isolates from bovine mastitis in Brazil (Duarte et al., 2005). All isolates also carried *erm*(B) and 6 of them *erm*(A). A recent study from Australia on *Strep. uberis* from dairy herds found a new gene, *mel* or *mef*(A), as well as *mrs*E (Vezina et al., 2021).

Acquisition of an *mph* gene confers resistance to macrolides only through the production of inactivating enzymes (Chesneau et al., 2007). Emergence of *mph*(B) has been documented in French *Strep. uberis* isolates from clinical mastitis cows (Achard et al., 2008). Finally, resistance to lincosamide only is achieved through genes that code for inactivating enzymes that adenylate these compounds on position 3 or 4. These are mainly from the *lnu* family. *lnu*A has been detected in *Strep. dysgalactiae* and *Strep. agalactiae* from Egypt, or *Strep. uberis* and *Strep. dysgalactiae* from Poland for example (Kaczorek et al., 2017; Ahmed et al., 2020). However, *lnu*B (also known as *lin*B) is by far the most common gene (Haenni et al., 2018). It has been found in *Strep. uberis* and *Strep. dysgalactiae* subsp. *dysgalactiae* and *Strep. agalactiae* (Schmitt-van de Leemput and Zadoks, 2007; Rato et al., 2013; Vélez et al., 2017; Reyes et al., 2019; Hernandez et al., 2021). *lnu*(D) has been detected in *Strep. uberis* from France and *lnu*(C) in *Strep. uberis* from Australia (Petinaki et al., 2008; Haenni et al., 2011; Vezina et al., 2021).

***Resistance to Tetracyclines.*** A slow but continuous increase of resistance to tetracycline in *Strep. uberis* from French dairy farms was reported between 2006 and 2016 (Boireau et al., 2018). Levels of resistance vary between streptococcal species, with *Strep. dysgalactiae* commonly showing a higher resistance prevalence (Mevius et al., 2008; Persson et al., 2011; Cameron et al., 2016; de Jong et al., 2018). Genes involved include membrane efflux systems [*tet*(K) *tet*(L)] and ribosomal protection enzymes [*tet*(M), *tet*(O), *tet*(S) (Haenni et al., 2018)]. Overall, *tet*(M) is the most commonly found although all 5 genes have been detected in *Strep. uberis*, *Strep. agalactiae*, or *Strep. dysgalactiae* from France, Brazil, Canada, Portugal, Poland, Egypt, Argentina, or China, for example (Duarte et al., 2005; Haenni et al., 2010; Rato et al., 2013; Kaczorek et al., 2017; Vélez et al., 2017; Reyes et al., 2019; Tian et al., 2019; Ahmed et al., 2020; Hernandez et al., 2021).

**Table 2.** Percent of resistance of *Streptococcus* from bovine mastitis to various antimicrobials in EU countries[1]

| Country | T[2] | Disc[3] or MIC | Standard[4] | Pen | Amp | Amc | Fur | Chl | Ery | Tyl | Pir | Lin | Cli | Spc | Gen | kan | Neo | Str | Cip | Enr | Tet | Smx | Tmp | Sxt | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Streptococcus uberis* | | | | | | | | | | | | | | | | | | | | | | | | | |
| Austria (n = 124) | S | Disc | CLSI | 2 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Baumgartner et al., 2018 |
| Belgium (n = 939) | D | Disc | CLSI | — | 1.6 | 0.1 | — | — | 30.4 | — | — | — | — | — | — | — | — | — | — | — | 40.8 | — | — | 2.5 | Supré et al., 2014 |
| Czech Republic (n = 163) | S | MIC | CLSI/ EUCASTT/ CA-SFM | 0 | — | 1.2 | 1.8 | — | — | — | — | — | 30.1 | — | 0 | — | — | 52.1 | — | 2.5 | 63.2 | — | — | 0 | Šlosárková et al., 2019 |
| Denmark (n = 61) | D | MIC | CLSI/EUCAST | 0 | — | — | — | 0 | 6.6 | — | — | — | — | 3.3 | — | — | — | 98.4 | 0 | — | 21.3 | — | 1.6 | 0 | Chehabi et al., 2019 |
| England and Wales (n = 44) | D | Disc | BSAC/AHVLA | 0 | 0 | 0 | — | — | — | 2.3 | — | — | — | — | — | — | 39.5 | — | — | — | 34.1 | — | — | — | UK-VARSS, 2020 |
| Finland (n = 89) | S | MIC | NCCLS/ SVARM | 0 | — | — | — | — | 0 | — | — | — | 0 | — | — | — | — | — | — | — | 1.1 | — | — | 0 | Pitkälä et al., 2004 |
| France* (n = 969) | D | Disc | CA-SFM | — | — | — | — | — | 14 | 18 | — | 15 | — | — | 2 | 6 | — | 12 | — | 32 | 16 | — | — | 10 | Anses, 2021 |
| Ireland (n = 165) | D | Disc | CLSI | 0 | 0 | — | 0 | — | 15.2 | — | 22.2 | — | — | — | — | — | — | — | — | — | 11.5 | — | — | — | DAFM et al., 2021 |
| Lithuania (n = 25) | S | Disc | CLSI | 12 | 12 | 8 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Klimiene et al., 2011 |
| Poland (n = 53) | S | MIC | CLSI | 0 | — | — | — | — | 6 | — | — | — | — | — | 96 | 83 | — | — | — | 0 | 34 | — | — | — | Kaczorek et al., 2017 |
| Portugal (n = 30) | S | Disc | CLSI | 0 | — | 0 | — | 0 | 26.7 | — | 53.3 | — | — | — | 80 | — | — | 100 | — | — | 60 | — | — | — | Rato et al., 2013 |
| Slovakia (n = 57) | S | Disc | CLSI/EUCAST | 10.5 | — | 3.5 | 5.3 | — | — | — | — | — | — | — | — | — | 74 | 78.9 | — | — | 8.8 | — | — | 5.3 | Holko et al., 2019 |
| Sweden (n = 89) | S | MIC | EUCAST | 0 | — | — | — | — | 0 | — | — | — | 0 | — | — | — | — | — | 0 | — | 22.2 | — | — | — | Duse et al., 2021 |
| Switzerland (n = 221) | D | Disc | CLSI | 7.8 | 7.7 | 0.4 | — | — | — | — | — | 49.7 | — | 36.2 | 99.5 | — | — | — | — | — | — | — | — | — | Rüegsegger et al., 2014 |
| *Streptococcus dysgalactiae* | | | | | | | | | | | | | | | | | | | | | | | | | |
| Austria (n = 35) | S | Disc | CLSI | 0 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Baumgartner et al., 2018 |
| Belgium* (n = 444) | D | Disc | CLSI | — | 0.7 | 0.2 | — | — | 19.5 | — | — | — | — | — | — | — | — | — | — | — | 93.2 | — | — | 1.4 | Supré et al., 2014 |
| Czech Republic (n = 25) | S | MIC | CLSI/ EUCASTT/ CA-SFM | 0 | — | 0 | 0 | — | — | — | — | — | 12 | — | 0 | — | — | 28 | — | 0 | 60 | — | — | 0 | Šlosárková et al., 2019 |
| Denmark (n = 33) | D | MIC | CLSI/EUCAST | 0 | — | — | — | 0 | 6.6 | — | — | — | — | 0 | — | — | — | 12.1 | 9.1 | — | 9.1 | — | 0 | 0 | Chehabi et al., 2019 |
| France* (n = 167) | D | Disc | CA-SFM | — | — | — | — | — | 11 | 14 | — | 11 | — | — | 0 | 7 | — | 5 | — | 48 | 88 | — | — | — | Anses, 2021 |
| Ireland (n = 52) | D | Disc | CLSI | 0 | 0 | — | 0 | | 1.9 | — | 3.8 | — | — | — | — | — | — | — | — | — | 57.7 | — | — | — | DAFM et al., 2021 |
| Lithuania (n = 30) | S | Disc | CLSI | 23.3 | 26.6- | 6.7 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Klimiene et al., 2011 |
| Poland (n = 41) | S | MIC | CLSI | 0 | — | — | — | — | 22 | — | — | — | — | — | 68 | 51 | — | — | — | 5 | 61 | — | — | — | Kaczorek et al., 2017 |
| Portugal (n = 18) | S | Disc | CLSI | 0 | — | 0 | — | 0 | 22.2 | — | 38.9 | — | — | — | 38.9 | — | — | 77.8 | — | — | 100 | — | — | — | Rato et al., 2013 |
| England and Wales (n = 21) | D | Disc | BSAC/AHVLA | 0 | 0 | 0 | — | — | — | 4.8 | — | — | — | — | — | — | 5.3 | — | — | — | 90.5 | — | — | — | UK-VARSS, 2020 |
| Sweden (n = 120) | S | MIC | EUCAST | — | — | — | — | — | — | — | — | — | 0 | — | — | — | — | — | — | — | — | — | — | — | Duse et al., 2021 |
| Switzerland (n = 221) | D | Disc | CLSI | 7 | 5.2 | 0 | — | — | — | — | — | 37.6 | — | 39.9 | 97.5 | — | — | — | — | — | — | — | — | — | Rüegsegger et al., 2014 |

*Continued*

**Table 2 (Continued).** Percent of resistance of *Streptococcus* from bovine mastitis to various antimicrobials in EU countries[1]

| Country | T[2] | Disc[3] or MIC | Standard[4] | Pen | Amp | Amc | Fur | Chl | Ery | Tyl | Pir | Lin | Cli | Spc | Gen | kan | Neo | Str | Cip | Enr | Tet | Smx | Tmp | Sxt | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Streptococcus agalactiae* | | | | | | | | | | | | | | | | | | | | | | | | | |
| Croatia* (n = 33) | D | Disc | CLSI | 36.4 | — | 57.8 | — | — | — | — | — | 33.3 | — | — | — | — | — | — | — | 36.4 | 78.8*** | — | — | 63.6 | Sukalić et al., 2021 |
| Denmark (n = 13) | D | MIC | CLSI/EUCAST | 0 | — | — | — | — | 0 | — | — | — | — | 0 | — | — | — | 100 | 0 | — | 76.9 | — | 0 | 0 | Chehabi et al., 2019 |
| Lithuania (n = 134) | S | Disc | CLSI | 28.3 | 46.3 | 16.4 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | Klimiene et al., 2011 |
| Poland (n = 27) | S | MIC | CLSI | 0 | — | — | — | — | 7 | | — | — | — | — | 100 | 96 | — | — | — | 0 | 44 | — | — | — | Kaczorek et al., 2017 |
| Portugal (n = 60) | S | Disc | CLSI | 0 | — | 0 | — | 0 | 18.3 | | 18.3 | — | — | — | 93.3 | — | — | −96.7 | — | — | 56.7 | — | — | — | Rato et al., 2013 |
| Slovakia (n = 30) | S | Disc | CLSI/EUCAST | 23.3 | — | — | — | — | — | — | — | 6.7 | — | — | — | — | 80 | 70 | — | 23.3 | 13.3 | — | — | — | Holko et al., 2019 |
| Ukraine (n = 43) | D | Disc | CLSI | — | — | — | 25.58 | — | — | — | — | — | — | — | — | — | — | — | — | — | 80 | — | — | — | Elias et al., 2020 |

[1]Pen: penicillin; Amp: ampicillin; Amc: amoxicillin + clavulanic acid; Fur: ceftiofur; Chl: chloramphenicol; Ery: Erythromycin; Tyl: tylosin; Pir: pirlimycin; Lin: lincomycin; Cli: clindamycin; Spc: spectinomycin; Gen: gentamicin; Kan: kanamycin; Neo: neomycin; Str: streptomycin; Cip: ciprofloxacin; Enr: enrofloxacin; Tet: tetracycline; Smx: sulfamethoxazole; Tmp: trimethoprim; Sxt: sulfamethoxazole + trimethoprim.

[2]T = type: D, diagnostic; S, survey.

[3]Disc = disc diffusion.

[4]CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; CA-SFM: Comité de l'Antibiogramme–Société Française de Microbiologie; BSAC: British Society for Antimicrobial Chemotherapy; AHVLA: Animal Health and Veterinary Laboratories Agency.

*Percent resistance was calculated using the % susceptibility published.

***Oxacillin was tested.

***Resistance to β-Lactams.*** Resistance to β-lactam antimicrobials in streptococci from bovine mastitis is usually low mainly because they cannot successfully acquire exogenous β-lactam resistance genes. However, decreased susceptibility, and in some cases resistance, has been detected in certain studies in low numbers (Guérin-Faublée et al., 2002; Tenhagen et al., 2006; Haenni et al., 2018). Resistance in *Strep. uberis* can be acquired by mutation (substitutions) in the penicillin-binding proteins (McDougall et al., 2020). This is how traditionally *Streptococcus* were thought to acquire β-lactam resistance. However, recent studies from Canada showed a higher prevalence of penicillin and ampicillin resistance in *Strep. uberis* and revealed the presence of the gene *bl2b* in *Strep. uberis* and *Strep. dysgalactiae* as well as TEM genes in the 2 species (TEM-1, TEM-127, TEM-136, TEM-157, TEM-163, TEM-47, TEM-89, and TEM-95 in *Strep. uberis* and TEM-71, TEM-1, TEM-136, TEM-157, and TEM-47 in *Strep. dysgalactiae*; Vélez et al., 2017). However, phenotypic resistance was not always observed when they were present (Reyes et al., 2019). The gene *blaZ* has been detected in *Strep. uberis* and *Strep. dysgalactiae* from Poland, but most isolates showed phenotypic susceptibility to penicillin (Kaczorek et al., 2017). In China, correlation between phenotypic resistance and the presence of a β-lactam resistance gene was low (3.13%), with higher numbers of phenotypic resistance (Tian et al., 2019). The relevance and potential of these β-lactamases have therefore yet to be elucidated in streptococcal species.

***Resistance to Other Antimicrobials.*** With regard to fluoroquinolones, resistance is mediated by point mutations in the quinolone resistance determinant regions of the *gyr*A and *par*C genes (Drlica and Zhao, 1997). These have been found in *Streptococcus* spp. from bovine mastitis in China (Zhang et al., 2018; Tian et al., 2019). Fifteen isolates presented quinolone resistance while only 9 carried resistance genes. Tian et al. (2019) also found higher phenotypic than genetic prevalence of resistance for chloramphenicol (*cat*1, *cat*2), or sulfonamides (*sul*1, *sul*2, *sul*3), with the latest showing the highest correlation between both.

Aminoglycoside resistance genes such as *aph*A-3 and *aad*-6 have been detected in *Strep. uberis* and *Strep. dysgalactiae* although in low numbers (Kaczorek et al., 2017; Ahmed et al., 2020). Again, phenotypic resistance levels were present in a higher number of isolates due to these species showing a naturally lower susceptibility to this group of antibiotics. Finally, resistance to streptogramin A has been identified in *Strep. uberis* via the *vat*D gene (Vezina et al., 2021).

## GENETIC DETERMINANTS OF RESISTANCE IN MASTITIS PATHOGENS: GRAM-NEGATIVE MICROORGANISMS

### *Enterobacteriaceae: E. coli and K. pneumoniae*

*Escherichia coli* is an opportunistic pathogen and the most frequent gram-negative bacterium responsible for bovine mastitis. It can cause IMI in cattle particularly during parturition or early lactation due to the immunosuppression taking place at these stages. Infections can result in severe clinical mastitis and sometimes become recurrent, although they are usually of shorter duration than those produced by other pathogens and a high number of them do not need treatment (Blowey and Edmondson, 2010). Although less commonly isolated, *K. pneumoniae* can also cause severe clinical mastitis inducing massive inflammation and necrosis of the mammary gland (Schukken et al., 2012).

*Enterobacteriaceae* are considered intrinsically resistant to macrolides, aminocoumarins or glycopeptides due to the poor membrane permeability of these antimicrobial classes, unable to penetrate the outer membrane of gram-negative bacteria (Klobucar and Brown, 2022). However, the macrolide azithromycin is associated with successful treatment (Gomes et al., 2017). AmpC enzymes can be chromosomal or plasmid-determined (Thomson, 2010). Chromosomically encoded AmpC genes in *E. coli* from milk have been detected although resistance is not always present due to low expression. For example, Fazel et al. (2019) detected $bla_{\text{ampC}}$ in more than 95% of *E. coli* isolates from clinical mastitis cases in Iran, although only 66% showed phenotypic resistance to ampicillin (Fazel et al., 2019). Similarly, Koovapra et al. (2016), detected $bla_{\text{ampC}}$ in 20 *K. pneumoniae* from bovine mastitis milk in India, but only 7 were positive phenotypically (Koovapra et al., 2016). In addition, *K. pneumoniae* produces small amounts of SHV β-lactamases, which can generate an intrinsic resistance to ampicillin and other β-lactams such as carbencillin or ticarcillin (Fu et al., 2007).

*Escherichia coli* and *K. pneumoniae* isolates are important from a public health and surveillance perspective as they can act as reservoirs for antimicrobial resistance genes. Acquired resistance in *Enterobacteriaceae* from mastitis is summarized below (Table 3).

***Resistance to β-Lactams.*** Resistance to β-lactams is possibly the most found in *E. coli* and *K. pneumoniae* isolates from bovine mastitis. β-Lactamases are enzymes that can hydrolyze chemical compounds with a β-lactam ring. However, a wide range of enzymes employ different hydrolyzing mechanisms and have dis-

**Table 3.** Percent of resistance of *Escherichia coli* and *Klebsiella pneumoniae* from bovine mastitis to various antimicrobials in EU countries[1]

| Country | Type[2] | Disc[3] or MIC | Standard[4] | Amp | Amc | Fur | Ctx | Chl | Ery | Col | Nal | Spc | Gen | kan | Neo | Str | Cip | Enr | Tet | Smx | Tmp | Sxt | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | | | | | | | | | | | | | | | | | | | | | | | |
| Belgium (n = 563) | D | Disc | CLSI | 28.8 | 7.3 | — | — | — | — | — | — | — | — | — | — | — | — | — | 14.7 | — | — | 9.6 | Supré et al., 2014 |
| Croatia* (n = 93) | D | Disc | CLSI | — | 45.5 | — | — | — | — | — | — | — | 82.8 | — | — | — | — | 19.4 | 78.5*** | — | — | 69.9 | Sukalić et al., 2021 |
| Czech Republic (n = 243) | S | Disc | CLSI | 30.4 | 0.4 | | 0.7 | | | 0 | 1.5 | | 0.4 | 1.6 | | 5.9 | 0.7 | | 13 | | | 3.3 | Skočková et al., 2015 |
| Denmark (n = 62) | D | MIC | CLSI/ EUCAST | 11.3 | 0 | 0 | 0 | 1.6 | — | 0 | 1.6 | 1.6 | 0 | — | 0 | 12.9 | 0 | — | 11.3 | 17.7 | 16.1 | — | Chehabi et al., 2019 |
| Finland (n = 144) | S | MIC | EUCAST | 18.7 | — | 0 | 1.4 | 6.9 | — | — | 0.7 | — | 0 | 6.3 | — | 18.1 | 0.7 | — | 16.7 | 14.6 | 10.4 | — | Suojala et al., 2011 |
| France (n = 1,114) | D | Disc | CA-SFM | — | 22 | 0 | — | 30 | — | — | 5 | — | 2 | 11 | 14 | — | — | 2 | 20 | 18 | 11 | 12 | Anses, 2021 |
| Germany (n = 224) | D | MIC | CLSI | 12.1 | 0.4 | 4.5 | — | — | — | — | — | — | 0.9 | — | — | — | 2.2 | — | 8.5 | — | — | 8.5 | BVL, 2018 |
| Ireland (n = 148) | D | Disc | CLSI | 18.2 | 4.1 | 0 | — | — | — | — | — | — | — | 6.1 | — | 16.9 | — | 5.4 | 12.2 | — | — | 11.5 | DAFM et al., 2021 |
| Italy (n = 105) | S | MIC | CLSI | 70 | 16 | — | — | 23 | — | — | — | — | 7 | — | — | — | — | 14 | 60 | — | — | 42 | Locatelli et al., 2019 |
| Portugal (n = 5,916) | D | Disc | CLSI | 99.4 | 30.3 | — | — | — | — | — | — | — | 3.6 | — | — | — | — | — | — | — | — | 13.1 | Rocha et al., 2014 |
| Slovakia (n = 65) | S | Disc | CLSI/ EUCAST | — | 22.1 | — | — | — | — | — | — | — | — | — | 24.7 | 35.1 | — | — | 2.6 | — | — | — | Holko et al., 2019 |
| Sweden (n = 116) | D | MIC | EUCAST | 8.6 | — | — | 4.3 | 0 | — | 6 | — | — | 0.9 | 2.3 | — | 7.8 | 1.7 | 0 | 4.3 | 6.9 | 3.5 | — | Duse et al., 2021 |
| Switzerland (n = 82) | D | Disc | CLSI | 14.7 | 1.6 | 0.8 | — | — | — | — | — | — | 2.5 | — | — | — | — | — | — | — | — | 6.6 | Nüesch-Inderbinen et al., 2019 |
| UK–England and Wales (n = 55) | D | Disc | BSAC/ AHVLA | 45.5 | 7.3 | — | — | — | — | — | — | — | — | — | 5.5 | 12.7 | — | 1.8 | 14.5 | — | — | 10.9 | UK-VARSS, 2020 |
| UK–Scotland (n = 118) | D | Disc | BSAC/ AHVLA | 22.9 | 9.3 | — | — | — | — | — | — | — | — | — | 1.7 | 15.3 | — | 2.5 | 16.9 | — | — | 11.9 | UK-VARSS, 2020 |
| Ukraine (n = 59) | D | Disc | CLSI | — | — | 43.26 | 56.25 | — | — | — | — | — | 26.27 | — | — | — | — | — | 18.75 | — | — | — | Elias et al., 2020 |
| *K. pneumoniae* | | | | | | | | | | | | | | | | | | | | | | | |
| Belgium (n = 59) | D | Disc | CLSI | — | 1.7 | — | — | — | — | — | — | — | — | — | — | — | — | — | 16.9 | — | — | 3.4 | Supré et al., 2014 |
| France (n = 73) | D | Disc | CA-SFM | — | 12 | 0 | — | — | — | — | 5 | — | 1 | 0 | 2 | 17 | — | 0 | 12 | — | — | 4 | Anses, 2021 |
| Denmark (n = 18) | D | MIC | CLSI/ EUCAST | 83.3 | 0 | 0 | 0 | 0 | — | 0 | 0 | 0 | 0 | — | 0 | 5.6 | 0 | — | 0 | 0 | 0 | — | Chehabi et al., 2019 |
| England and Wales (n = 12) | D | Disc | BSAC/ AHVLA | 100 | 0 | — | — | — | — | — | — | — | — | — | 0 | 0 | — | 0 | 8.3 | — | — | 0 | UK-VARSS, 2020 |
| Portugal (n = 773) | D | Disc | CLSI | 100 | 37.4 | — | — | — | — | — | — | — | 1.3 | — | — | — | — | — | — | — | — | 6.1 | Rocha et al., 2014 |
| Sweden (n = 45) | S | MIC | EUCAST | 95.4 | — | — | 0 | — | — | 4.6 | — | — | — | — | — | — | 4.6 | — | 9.1 | — | — | 0 | Duse et al., 2021 |

[1]Amp: ampicillin; Amc: amoxicillin + clavulanic acid; Fur: ceftiofur; Ctx: cefotaxime; Chl: chloramphenicol; Ery: Erythromycin; Col: colistin; Nal: nalidixic acid; Spc: spectinomycin; Gen: gentamicin; Kan: kanamycin; Neo: neomycin; Str: streptomycin; Cip: ciprofloxacin; Enr: enrofloxacin; Tet: tetracycline; Smx: sulfamethoxazole; Tmp: trimethoprim; Sxt: sulfamethoxazole + trimethoprim.

[2]T = type: D, diagnostic; S, survey.

[3]Disc = disc diffusion.

[4]CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; CA-SFM: Comité de l'Antibiogramme–Société Française de Microbiologie; BSAC: British Society for Antimicrobial Chemotherapy; AHVLA: Animal Health and Veterinary Laboratories Agency.

*Percent resistance was calculated using the % susceptibility published.

***Oxacillin was tested.

tinct functional capabilities (Bush, 2018). TEM-1 and SHV-1 and SHV-2 β-lactamases inactivate penicillins and narrow-spectrum cephalosporins (EFSA, 2011). Their success is probably due to their easy dissemination through plasmids and other mobile genetic elements (Tooke et al., 2019). The gene $bla_{\text{TEM-1}}$ has been shown to be present in mastitis *E. coli* isolates from China, Greece, or Canada for instance, and in *K. pneumoniae* from Egypt or the United Kingdom (Ahmed and Shimamoto, 2011; Timofte et al., 2014; Filioussis et al., 2020; Yu et al., 2020; Majumder et al., 2021). SHV-1 and 2 enzymes carried by plasmids are less common, although $bla_{\text{SHV-1}}$ was detected in *K. pneumoniae* mastitis strains from the United States or *E. coli* from China, and $bla_{\text{SHV-1}}$ and $bla_{\text{SHV-2a}}$ in *K. pneumoniae* from Indonesia (Sudarwanto et al., 2015; Ali et al., 2016; Zheng et al., 2021).

The emergence of these enzymes up until the 1960s increased the need for further development of new antimicrobials and led to the introduction of β-lactamase inhibitors in the 1970s, and third generation cephalosporins and carbapenems in the 1980s and 1990s (Padmini et al., 2017). However, extended spectrum β-lactamases (**ESBL**) and AmpC cephalosporinases started to emerge then mainly in *E. coli* and *K. pneumoniae* isolates increasing the incidence of nosocomial infections (Padmini et al., 2017). Extended spectrum β-lactamases can hydrolyze penicillins, first-, second-, third- and fourth-generation cephalosporins as well as monobactams such as aztreonam, but not cephamycins or carbapenems (Jacoby and Munoz-price, 2005). Additionally, they are susceptible to β-lactam inhibitors such as clavulanic acid, sulbactam, or tazobactam. The genes coding for these enzymes rarely integrate in the bacterial chromosome, and are associated with insertion sequences such as IS*Ecp1*, IS*CR1*, or IS*26*, Transposons such as Tn2 or integrons (Poirel et al., 2018). Nowadays about 150 different enzymes have been described from each of the CTX-M, SHV, or TEM families (ur Rahman et al., 2018). The $bla_{\text{CTX-M-15}}$ gene has been found in numerous mastitis *E. coli* and *K. pneumoniae* isolates from around the globe. Other examples for ESBL detected in *E. coli* include CTX-M-1, CTX-M-2, CTX-M-3, CTX-M-14, CTX-M-55, CTX-M-96, SHV-12 from Germany, Switzerland, Japan, France, or Colombia (Geser et al., 2012; Dahmen et al., 2013; Ohnishi et al., 2013; Ali et al., 2016; Vásquez-Jaramillo et al., 2017). In *K. pneumoniae* a wider range of SHV β-lactamases is present: such as SHV-11, SHV-12, SHV-27, SHV-28, SHV-52 SHV-61, SHV-83, SHV-98, SHV-108, SHV-148, and also CTX-M-1, CTX-M-2, CTX-M-8, and CTX-M-14, all from countries such as Japan, France, Italy, Indonesia, Egypt, or Brazil (Locatelli et al., 2010; Ahmed and Shimamoto, 2011; Dahmen et al., 2013; Ohnishi et al., 2013; Sudarwanto et al., 2015; Nobrega et al., 2021; Tsuka et al., 2021). Early OXA β-lactamases are also ESBL and have been detected in *E. coli* mastitis isolates in Lebanon (Evans and Amyes, 2014; Abboud et al., 2021).

In contrast, AmpC cephalosporinases hydrolyze cephalosporins and cephamycins and can resist inhibition by β-lactam inhibitors. Bacteria such as *E. coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, or *Serratia marcescens* possess a chromosomal AmpC that can be overproduced. Others such as *Klebsiella* spp. or *Salmonella* spp. can only acquire an AmpC enzyme by plasmid transfer (Philippon et al., 2002). AmpC are much less prevalent than ESBL, with plasmid-borne gene $bla_{\text{CMY-2}}$ having been detected in *E. coli* from bovine milk in Switzerland, Thailand, South Korea, or Lebanon for instance (Endimiani et al., 2012; Hinthong et al., 2017; Tark et al., 2017; Abboud et al., 2021). $bla_{\text{CMY-59}}$ was detected in *E. coli* isolated from mastitic dairy cattle in Canada (Majumder et al., 2021).

Finally, carbapenemase-producing *Enterobacteriaceae* can hydrolyze penicillins, all cephalosporins, monobactams, carbapenems, and β-lactamase inhibitors (Papp-Wallace et al., 2010). The wide spectrum of resistance of bacteria carrying these genes is of concern to public health as some have been shown to be susceptible only to colistin and tigecycline (Kumarasamy et al., 2010). *K. pneumoniae* was the first bacteria identified to produce carbapenemase enzymes (Munoz-Price et al., 2013). The name for this enzyme family was adopted from this first *K. pneumoniae* carbapenemase (KPC) outbreak, but nowadays several other gram-negative bacilli have been identified to carry the $bla_{\text{KPC}}$ gene. This is a transposon associated gene, capable of inserting in a plasmid (Arnold et al., 2011). Recently, *K. pneumoniae* from bovine mastitis samples in Mexico have been reported to produce carbapenemase KPC-2 (Silva-Sanchez et al., 2021). Other important carbapenemases include NDM, OXA-23, OXA-48, IMP, or VIM (Cui et al., 2019). The $bla_{\text{NDM-1}}$ gene has been detected in *E. coli* isolated from milk of cattle suffering from clinical or subclinical mastitis in India, whereas *K. pneumoniae* from milk samples from cows in Pakistan were detected to carry $bla_{\text{NDM-1}}$ and $bla_{\text{oxa-48}}$ (Ghatak et al., 2013; Chaudhry et al., 2020). The $bla_{\text{NDM-5}}$ gene has been identified in *E. coli* and *K. pneumoniae* milk samples from Algeria and China, respectively (Yaici et al., 2016; He et al., 2017).

***Resistance to Tetracyclines.*** Resistance to tetracyclines in *E. coli* from livestock is widespread due to tetracyclines being used widely in veterinary medicine. This is very likely the same in *K. pneumoniae*. The most prevalent genes are *tet*(A) and *tet*(B), which may be combined in the same isolate (Metzger and Hogan,

2013). They are part of nonconjugative transposons, Tn*1721* and Tn*10*, respectively, that are often integrated in plasmids (Poirel et al., 2018).

The *tet*(A) or *tet*(B) have been detected in Irish, Swiss, US, or German *E. coli* isolates and in Brazilian or Canadian *K. pneumoniae* isolates (Lanz et al., 2003; Srinivasan et al., 2007; Metzger and Hogan, 2013; Keane, 2016; Freitag et al., 2017; Massé et al., 2020; Majumder et al., 2021).

In a recent study from Jordan, 100% of *E. coli* isolates from bovine mastitis carried *tet*(B), *tet*(E), and *tet*(G) genes, 93% carried *tet*(A) and 71% *tet*(D) (Ismail and Abutarbush, 2020). In China distribution varies; when *tet*(A), *tet*(B) and *tet*(C) were screened in *E. coli* only *tet*(C) was detected, whereas another study only detected *tet*(A) when screening for *tet*(A) and *tet*(B) (Lan et al., 2020; Yu et al., 2020). In the United States, although *tet*(A) and *tet*(B) were present, *tet*(C) showed a higher prevalence (Metzger and Hogan, 2013). In a more recent US study on *K. pneumoniae*, genes *tet*(B) and *tet*(D) were detected (Zheng et al., 2021). In Iran, however, Jamali et al. (2018) detected *tet*(B), *tet*(A), *tet*(D), *tet*(E), and *tet*(C) (in order of decreasing %) from *E. coli* isolates (Jamali et al., 2018).

***Resistance to Aminoglycosides.*** Resistance to amynoglycosides such as neomycin, streptomycin, gentamicin, or kanamycin is widespread and can be acquired by 2 main mechanisms: target modification and enzymatic inactivation. The first 16S RNA methylase, ArmA, was reported in a human *K. pneumoniae* isolate in 2003. Posterior reported enzymes include RmtA/B/C/D/E/F/G/H and NmpA (Poirel et al., 2018). Very few have been reported in animals, with gene *rmt*B having been detected in *E. coli* and *K. pneumoniae* milk isolates from China (He et al., 2017; Yu et al., 2015).

Enzymatic modification is a more common resistance mechanism found in *Enterobacteriaceae*. There are several groups of acetyltransferases depending on the position of the drug structure where the acetyl group is added, resulting in probably hundreds of enzymes for which different nomenclatures exist that are sometimes inconsistent (Ramírez and Tolmasky, 2010). For this reason, we will only try to capture what each publication has reported. Aminoglycoside N-acetyltransferases AAC(3)-II/IV and AAC(6)-Ib are the most common acetyltransferases in *E. coli* and are usually part of gene cassettes in integrons (Poirel et al., 2018). Gene *aac*(6′)-Ib-cr variant can induce resistance against aminoglycoside and fluoroquinolones simultaneously (Kim et al., 2011). This has been detected in a *K. pneumoniae* isolate from Tunisia or *E. coli* from Germany for instance (Freitag et al., 2017; Saidani et al., 2018).

Nucleotidyltransferases ANT(2″) and ANT(3″) coded by the *aad*B and *aad*A genes are contained within gene cassettes in class 1 integrons where they usually co-exist combined with resistance genes for other antimicrobial classes. Majumder et al. (2021) found gene *aad*A2 present for the first time in Canadian cattle with mastitis. In China, there was a predominant combination of *dfr*A17-*aad*A5 genes in *E. coli* isolates, that confer resistance to aminoglycosides and trimethoprim (Ali et al., 2016). Koovapra et al. (2016) detected gene cassettes in class 1 integrons from *K. pneumoniae* isolates in India combining *dfr*A17 and *aad*A5 or *dfr*A12 and *aad*A2bq (Koovapra et al., 2016).

Similarly, phosphotransferases APH(6)-Ia and APH(6)-Id coded by *str*A and *str*B genes are usually found in animal isolates (Poirel et al., 2018). Both genes confer streptomycin resistance, are often present together both in *E. coli* and *K. pneumoniae*, and can sometimes be associated with *aph*(3″)-I/II genes conferring also kanamycin resistance (Lanz et al., 2003; Srinivasan et al., 2007; Fazel et al., 2019; Alawneh et al., 2020; Massé et al., 2020). This was the case for example in Ireland where a single *E. coli* isolate from bovine mastitis carried *str*A, *str*B, *aad*A1, and *aph*A genes and was resistant to streptomycin, neomycin, and kanamycin (Keane, 2016). Genes may be combined in many different ways in gene cassettes. For instance, Yang et al. (2021) reported a *K. pneumoniae* isolate carrying *aac*(6′)-Ib, *aph*(3′)-I, and *ant*(3″)-I in China. In this study, *aac*(6′)-II showed the highest prevalence (24.24%) within the aminoglycoside resistance genes (Yang et al., 2021).

***Resistance to Quinolones.*** As in other bacterial species, resistance to quinolones can be caused by mutations of the *gyr*A/*gyr*B or *par*C/*par*E chromosomal genes that code for gyrases and topoisomerases, respectively (Yang et al., 2018). From a total of 92 *E. coli* isolates from mastitis samples in China, 97.8% carried *gyr*A mutations, and 95.6% carried *gyr*B mutations (Lan et al., 2020). However, plasmid-borne quinolone resistance determinants seems to be a more frequent mechanism of quinolone resistance in mastitic bacteria, given the increased number of studies reporting on their presence. In animals, these are represented by Qnr such as proteins, the already mentioned gene AAC(6′)-Ib-cr acetyltransferase, or active efflux pumps such as QepA and OqxAB (Poirel et al., 2018). The *oqx*AB gene can also reduce susceptibility to other antimicrobial families such as trimethoprim and chloramphenicol, and was highly prevalent in *K. pneumoniae* from US, China, or Tunisia (Saidani et al., 2018; Kim et al., 2021; Zheng et al., 2021). Although less common in *E. coli*, Yang et al. (2018) detected *oqx*AB in 18% of isolates, together with *qep*A4, *qnr*S, and *qnr*B2 that were present at a lower prevalence (Yang et al., 2018). Other genes encountered in *E. coli* include *qnr*B or *qnr*A, and although

they are not as widely spread as studies from Brazil, Germany and Jordan did not find any of these (Freitag et al., 2017; Saidani et al., 2018; Ismail and Abutarbush, 2020; Nobrega et al., 2021). It is not unusual to find ciprofloxacin resistance linked to nalidixic acid resistance in *E. coli* isolates, which is mostly due to mutations in *gyr*A or *par*C genes (Balakrishnan et al., 2016). In *K. pneumoniae*, the *qnr*5, *qnr*S, *qnr*B, *qnr*A, *qnr*A1, or *qnr*S1 gene has been detected in Egypt or China (Ahmed and Shimamoto, 2011; He et al., 2017). Similar results were found by Koovapra et al. (2016) in India, where *K. pneumoniae* isolates were also tested for *qep*A, which was not present.

***Resistance to Sulfonamides.*** The most common sulfonamide resistance genes are *sul*1, *sul*2, and *sul*3. These are also carried in plasmids that can harbor other antimicrobial resistance genes and are widely disseminated. Genes *sul*1 or *sul*2 are the most commonly found in isolates from bovine mastitis and were present in *K. pneumoniae* from Brazil or Canada and *E. coli* from Germany, Ireland, Switzerland, or Australia (Lanz et al., 2003; Keane, 2016; Freitag et al., 2017; Alawneh et al., 2020; Massé et al., 2020; Nobrega et al., 2021). Resistance can also be present by mutations on the *fol*P gene, as was recently found in the United States (Zheng et al., 2021).

***Resistance to Trimethoprim.*** Numerous *dfr* genes have been detected in *Enterobacteriaceae* from bovine mastitis. Most of these are located in gene cassettes inserted into integrons class 1 or 2. The most usual genes that have been isolated in *K. pneumoniae* and *E. coli* from mastitis cases worldwide are *dfr*A1, *dfr*A5, *dfr*A7, *dfr*A12, *dfr*A15, *dfr*A16, and *dfr*A17 (Ahmed and Shimamoto, 2011; Keane, 2016; Koovapra et al., 2016; Freitag et al., 2017; Majumder et al., 2021; Nobrega et al., 2021).

***Resistance to Other Antimicrobials.*** Resistance to other antimicrobial classes that are of particular importance, such as colistin, have also been detected in *Enterobacteriaceae* from mastitis. The colistin resistance gene *mcr*-1 has been detected in *E. coli* from China and Greece (Filioussis et al., 2020; Liu et al., 2020). Additionally, Kieffer et al. (2015) found that in *K. pneumoniae* isolated from bovine mastitis in France, the inactivation of the *mgr*B gene caused colistin resistance, a mechanism already described in human, but not animal isolates until then (Kieffer et al., 2015). Acquired resistance to macrolides has also been shown with *mphA* and has been detected in *K. pneumoniae* from the United States or *E. coli* from Ireland, and ereA2 in *E. coli* from Egypt (Ahmed and Shimamoto, 2011; Keane, 2016; Zheng et al., 2021). Finally, *cm*1A, *cat*A1, *cat*A2, and *flo*R genes are associated with chloramphenicol resistance and all except for *cm*1A, which was only detected in *K. pneumoniae* from Brazil, have been reported from both bacterial species (Ahmed and Shimamoto, 2011; Keane, 2016; Lan et al., 2020; Nobrega et al., 2021).

## PHENOTYPIC PREVALENCE OF AMR IN EUROPE

Resistance levels of mastitis pathogens in European countries vary greatly. For instance, studies reporting MIC have shown resistance to penicillins in 31–47% *Staph. aureus* isolates from Germany (Tenhagen et al., 2006), 10–24.3% from the Netherlands (Hendriksen et al., 2008), or 7.1% from Sweden (Bengtsson et al., 2009). However, it is important to stress that comparison between countries is not always possible, as different laboratory methods or result interpretation standards are used. Interpretation of results is usually done by applying international breakpoint values that have been developed by trusted organizations. These include Clinical and Laboratory Standards Institute (CLSI), which is a volunteer-driven, membership-supported, not-for-profit, standards-development organization that provides clinical breakpoints that can be used to inform prescribing. The European Committee on Antimicrobial Susceptibility Testing (EUCAST), however, is a standing committee jointly organized by ESCMID, ECDC, and European national breakpoint committees. They provide both ECOFF (epidemiological cut-off breakpoints) and clinical breakpoints. Some research studies may develop their own breakpoints by using the distribution of their results and a wild type population (Supré et al., 2014).

Some studies may perform sample size and distribution analyses previously to obtain a representative sample of the population. However, others employ isolates or data sets that originate from diagnostic samples being submitted for testing, which may result in an over-representation of problematic herds or individuals. In the following tables, we aim to compare resistance in EU countries, but to account for these factors we have included the type of study, laboratory method, and standard followed. Publications where resistance was calculated as a percentage were included. More recent publications from these countries may be available, although they were not included because percent resistance was not provided, or it was calculated for a group of species.

## THE FUTURE OF AMR SURVEILLANCE IN EUROPE: VETERINARY MEDICINES AND POLICY

Antimicrobial resistance was responsible for about 33,100 deaths in the EU/EEA in 2015, and it is estimated that by 2030 resistance to second-line antibiotics

will be 72% higher compared with 2005 (Cassini et al., 2019; ECDC, 2019). The World Health Organization (WHO) led the publication in 2015 for a Global Action Plan on Antimicrobial Resistance. The Food and Agriculture Organization (FAO) and the World Health Organization for Animal Health (OIE) collaborate with WHO to implement relevant strategies and take part in an international consensus through the One Health initiative. This alliance advocates for actions across the human health, animal health, and environmental sectors to tackle the global threat AMR represents. Within the action plan, an objective is to strengthen the knowledge and evidence base through surveillance and research (WHO, 2015).

The EU introduced the mandatory surveillance of AMR in zoonotic bacteria (*Salmonella* and *Campylobacter*) and indicator bacteria (*E. coli*) from healthy food-producing animals (cattle, poultry, pigs) with Directive 2003/99/EC and Implementing Decision 2013/652/EU, which has now been repealed by Implementing Decision (EU) 2020/1729.

A harmonized surveillance of veterinary clinical isolates is, however, not yet performed in the European Union. Several European countries have surveyed AMR from veterinary clinical isolates as part of their AMR National Plans for some years now. This includes the Netherlands, Sweden, Denmark, Germany, France, and the United Kingdom (Mevius et al., 2009; BVL, 2018; Anses, 2019; Swedres-Svarm, 2019; UK-VARSS, 2019; SSI, 2020). The Netherlands or France, for example, have been monitoring for longer, although the Netherlands stopped reporting data on streptococci from isolates collected from milk in 2008. The surveillance of resistance from these isolates is of great importance to animal health and welfare, and to public health due to the role of these bacteria as antimicrobial resistance gene reservoirs.

A lack of international harmonization in the investigation of veterinary AMR has led to variations in the methods employed with regard to laboratory methods (culture, and the use of disc diffusion, MIC, Vitek) and the standards followed for interpretation (CLSI, EUCAST, CA-SFM, AHVLA, and so on), making comparisons between countries challenging. Surveillance of AMR in veterinary medicine, therefore, still has some aspects that need to be addressed.

Since 2017, the EU Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI; https://eu-jamrai.eu) offers support to all EU countries on the best practices and policies for the correct implementation of national plans. This is done by adopting the new European One Health Action Plan against AMR (European Commission, 2017), which builds on improvements to the previous action plan approved in 2011. From this project emerged the European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-Vet), which brings together professionals working in the surveillance of AMR in diseased animals within the EU aiming to establish best practices and standardize and harmonize AST in the veterinary field (Mader et al., 2022).

The EU commission requested the European Medicines Agency (EMA) and Antimicrobial Advice Ad Hoc Expert Group (AMEG) provide scientific advice on the effect of using antibiotics in animals on public health and animal health, and measures to manage the possible risk to humans. The EMA categorized antibiotics according to their risk to support veterinarians in their choice for treatment in 2014, which was later updated in 2020 (https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/advice-impacts-using-antimicrobials-animals). The need to reduce the use of antimicrobials to stop the development of new resistant strains has resulted in a new EU policy, Regulation (EU) 2019/6 on veterinary medicinal products and Regulation (EU) 2019/4 on medicated feed, which will be applied on January 28, 2022 (European Parliament and the Council of the European Union, 2019a,b). This new legislation aims to reduce the use of antimicrobials in the animal health sector by introducing new restrictions, and the possibility to reserve certain antimicrobials for human use only. This situation is predicted to encourage veterinary professionals to perform an antimicrobial sensitivity test before prescribing certain drugs of a higher categorization. This will allow for an informed treatment that maximizes therapeutic efficacy and justifies the use of a particular antimicrobial. This will result in an increased need for overall laboratory and testing capacity, both from private and governmental institutions. The lack of veterinary clinical breakpoints for several antimicrobials-pathogen combinations is a challenge that must be addressed to ensure harmonization of testing and interpretation, most importantly because this will be a key tool in the near future for prescribing by veterinary practitioners. Furthermore, although biased due to its diagnostic nature, this testing may generate valuable data on veterinary pathogens and their AMR in the EU.

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