Chapter

Antimicrobial Resistance of Cattle Mastitis-Causing Bacteria: How to Treat?

Zinka Maksimović, Benjamin Čengić, Amel Ćutuk and Alan Maksimović

Abstract

Cattle mastitis continues to be a global burden for the dairy industry, and its control depends on preventive measures, rapid detection and identification of involved pathogens and accurate antimicrobial treatment. The (mis)use of antimicrobials initiated a rapid evolutionary process of bacterial resistance by natural selection and led to the increased frequency and spread of bacterial antimicrobial resistance (AMR). The global AMR emergency and the prudent use of antimicrobials in cows have raised questions about alternative treatment approaches; however, the use of antimicrobials remains the principal method for mastitis therapy. This chapter summarise the current knowledge on AMR in cattle mastitis as a multifactorial global problem, the trends in AMR patterns in the most common mastitis-causing bacterial pathogens and altering factors, the policies and actions restricting the use of antimicrobials in cows and related challenges in the treatment. The reasons influencing the outcome of treating an intramammary infection, such as the selection of appropriate antimicrobial agents, optimal drug regimens, the gaps in antimicrobial susceptibility testing (AST) of mastitis pathogens and interpretation criteria, and the paradoxical relationship between antimicrobial *in vitro* activity and *in vivo* efficacy are discussed. The importance of effective mastitis control programmes is emphasised by an overview of (accurate) diagnosis, the evaluation of the therapy, cow health control and farm management practices.

Keywords: cattle mastitis, bacteria, antimicrobial resistance, treatment, intramammary infection

1. Introduction

Mastitis, defined as the inflammation of the mammary gland, is the most frequent disease of dairy cattle and a significant economic burden for the dairy industry worldwide, affecting health, well-being, milk production and reproduction efficiency of cows [1, 2]. The most common cause of mastitis is an intramammary infection [3]. This disease can present in a clinical and subclinical form [4]. Clinical mastitis is

characterised by visible abnormalities in the milk or in the udder, whereas subclinical mastitis is not visible and therefore is more difficult to detect. In addition, it occurs more frequently than clinical mastitis, and its duration is longer, which provides more opportunity for pathogens to spread between cows [3, 5, 6]. Depending on duration, mastitis occurs as peracute, acute, subacute and chronic [7]. Based on severity, it can be classified as mild (observable abnormalities in milk, generally clots or flakes, with little, or no signs of swelling of the mammary gland or systemic illness), moderate (visible abnormal milk accompanied by swelling in the affected mammary quarter with an absence of systemic signs of illness), and severe (sudden onset with grave systemic and local signs) [5, 8]. Severity and duration of mastitis mainly depend on the pathogen (s) involved, the host's health status/immune response and environmental factors [4]. Among various microorganisms associated with cattle mastitis, bacteria are the most frequently reported causative agents [9]. Traditionally and according to their primary source and transmission mode, mastitis causative agents have been classified as environmental pathogens (primary source is the habitat of the cow) and contagious pathogens (the main source is the mammary gland of infected cows) [7, 9, 10]. Furthermore, mastitis causative agents have been referred as major and minor pathogens, related to their prevalence, the severity of signs and the impact on cow health, milk quality and productivity [6, 11]. *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma bovis* are regarded as major contagious pathogens. Environmental pathogens are numerous: *Streptococcus uberis, S. equinus*, (*S. bovis*), (*S. dysgalactiae*), *Enterococcus* spp. (*Enterococcus faecalis*, *E. faecium, E. durans*), coliforms (*Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Enterobacter aerogenes*), non-coliforms (*Proteus* spp., *Serratia* spp., *Yersinia* spp.), and others (*Pseudomonas aeruginosa*, *Trueperella pyogenes*) [6]. Minor contagious pathogens include coagulase negative staphylococci (CoNS) (*S. chromogenes, S. haemolyticus, Staphylococcus epidermidis, S. simulans, S. sciuri*), and *Corynebacterium bovis* [6]. The classification of pathogens as contagious or environmental is misleading, particularly for *S. aureus*, *S. uberis*, *S. agalactiae* and other streptococcal species because of potentially multiple transmission routes associated with the strains [12, 13]. The prevalence, dominance and distribution of mastitis pathogens vary temporally, within and between herds and countries [2, 14]. The changing trends of mastitis causative agents induces shifts in major and minor pathogens [11, 14]. Currently, *S. aureus* (25%), CoNS (20%), *E. coli* (11%), *S. agalactiae* and *S. uberis* (9%) are recognised as the major mastitis causative agents [15]. The control of the disease is challenging due to the difficulties in its prevention, diagnosis and treatment, largely depending on the effects of national mastitis control programmes. Regardless of the control strategy, antimicrobial treatment of mastitis in dairy cattle is an established component of mastitis control programmes [16]. However, the use of antimicrobials in dairy cows can contribute to increased antimicrobial resistance (AMR) [17], which is one of the major challenges for bovine mastitis therapy. The development of antimicrobial resistance is an adaptive response of bacteria to antimicrobials as environmental threats and their inappropriate use [18]. Reservoirs of antimicrobial-resistant pathogens, antimicrobial residues, zoonotic pathogens and antimicrobial-resistant bacteria in milk are risk factors of concern for public health [19]. Cow health and welfare on dairy farms are compromised not only by various (antimicrobial-resistant) pathogens and often untreatable infections, but also by the limitations of antimicrobial susceptibility testing (AST) and interpretative criteria, and the restrictions on use of antimicrobials in food-producing animals due to the AMR crisis.

2. Cattle mastitis in the era of antimicrobial resistance

The global crisis of AMR is induced by increasing globalisation, the needs of the large human population, intensive food production and changing climate associated with the increasing frequency of AMR among microorganisms and development of complex survival strategies as evolutionary response under the pressure of the widespread, inappropriate and extensive use of antimicrobials [18, 20, 21]. Thus, the AMR circle is linked to (pathogenic) microorganisms, the use of antimicrobials in animals and humans and the environment. The threat of globally increasing AMR to animal and human health, followed by the limited development of new antimicrobials and resolutions has led to national and international activities and rigorous measures [22–25] to reduce using of antimicrobial agents. The resulting limitations and increased need to combat the infections and AMR pathogens have prompted the search for alternative solutions and potential substitutes for antimicrobials in mastitis treatment for dairy cows [26, 27]. As the response to AMR, A Global Action Plan on AMR [23] addressing the challenge of AMR through a "One Health" approach was issued in 2015 by the World Health Organisation (WHO) in collaboration with the World Organisation for Animal Health (WOAH) and the Food and Agriculture Organisation of the United Nations (FAO) [28]. More recently, the European Commission (EC) has adopted a proposal for a Council Recommendation on stepping up the European Union (EU) actions to combat AMR in a One Health approach. The objectives are: strengthen One Health national action plans on AMR; reinforce surveillance and monitoring of AMR and antimicrobial consumption; strengthen infection prevention and control; strengthen antimicrobial stewardship and prudent use of antimicrobials; recommend targets for AMR and antimicrobial consumption in human health; improve awareness, education and training; foster research and development, and incentives for innovation and access to antimicrobials and other AMR medical countermeasures; increase cooperation; and enhance global actions [29]. Mastitis (42%) and respiratory disease (20%) are the main indications for antimicrobial use in cattle in Europe [30, 31]. Considering an estimated 60–70% of all antimicrobials used on dairy farms are for preventing and treating mastitis [6], further efforts to improve mastitis control may significantly contribute to reduction in the use of antimicrobials [32]. The rules laid down in the Veterinary Medicines Regulation in the EU [33], which provide a wide range of measures to fight AMR, including the prudent and responsible use of antimicrobials in (food-producing) animals, the restrictions on prophylactic and metaphylactic use in animals, and reserving certain antimicrobials for the treatment of infections in people, have been applied from 2022. In response, pragmatic national and farm-level recommendations in support of improved mastitis control and intramammary antimicrobial stewardship in the Irish dairy industry have developed [32]. The measures applied in Denmark and the Netherlands showed substantial reducing on-farm antimicrobial usage over the last decade [32]. The shared actions included a ban on the prophylactic use of antimicrobials, a national database of antimicrobial usage allowing objective measurement and benchmarking and transparent reporting, clarity on the level of veterinary oversight required, detailed treatment guidelines, national reduction targets in antimicrobial usage, and restrictions on the usage of specific antimicrobials. Several antimicrobial agents used for mastitis therapy, categorised in Veterinary Critically Important Antimicrobial Agents (VCIA) or Veterinary Highly Important Antimicrobial Agents (VHIA) [25] are critically important for human health and

should not be as used a first-line treatment. Strict control and reducing inappropriate antimicrobial use in animals and humans are among the high priorities for addressing the AMR crisis; however reserving antimicrobials for human use only and limiting their use in veterinary medicine raise the question of the inability to treat infections in cows and consequences for cow health and welfare. Strategies that are more comprehensive should be promptly adopted in order to contain spread of infectious diseases among and between cows and humans, as policies to manage reservoirs of the pathogens related to cows, humans and the environment. A recent study [34] found a bidirectional association of antimicrobial consumption and AMR between humans and animals: animal antimicrobial consumption is positively linked with resistance in the human bacterial pathogens, while increased antimicrobial use in humans is associated to increased animal AMR. Moreover, socioeconomic factors play a significant role in the spread of AMR, implying antimicrobial consumption as a secondary risk factor, which reduction alone will not be sufficient to combat the worldwide AMR crisis [34]. This observation was supported by a more recent study [35], which showed that decrease in usage only slowly decreases resistance with no evidence of a reversal of resistance, and thus reducing usage is not a complete solution to alleviating high levels of resistance. Although antimicrobial consumption is considered as the most important factor contributing to AMR, resistance transmission appears to be the main driver for AMR levels [36].

Surveillance and monitoring of AMR is essential for assessing the trends related to the prevalence, source, spread and geographical distribution of AMR bacterial pathogens, for the early detection of emerged resistance and to provide information for evaluating antimicrobial usage and effects of actions to combat AMR [37]. National monitoring systems for AMR in bacterial pathogens of animals have been implemented by numerous countries [38–40]. Since the monitoring of AMR in bacterial pathogens of animals is not currently coordinated at European level, the European Union Joint Action on Antimicrobial Resistance and Healthcare Associated Infections (EU-JAMRAI) recently has recommended building the European Antimicrobial Resistance Surveillance network in Veterinary medicine (EARS-Vet) [39]. Despite numerous AMR surveillance systems on national and international level, such systems are still lacking in many countries. An online platform for surveys and maps of AMR in animals (resistancebank.org) that centralises information from low- and middle-income countries was recently introduced [40]. VetPath is an ongoing pan-European antimicrobial susceptibility monitoring programme collecting pathogens from diseased cattle, pigs and poultry not recently treated with antimicrobials [41]. The results of third VetPath monitoring period (2015–2016) for bacteria isolated pre-treatment from cows with acute clinical mastitis across European countries showed that mastitis pathogens were susceptible to most antimicrobials with exceptions of *S. aureus* (25.5%) and CoNS (29.1%) against penicillin, *S. uberis* against erythromycin (24%) and tetracycline (37.5%) and *S. dysgalactiae* against tetracycline (43.2%). High ampicillin and tetracycline resistance of 24% and 23.6%, respectively was observed in *E. coli*. The percentage resistance and the MIC values (the minimum inhibitory concentration-the lowest concentration of the antimicrobial required to prevent the replication of the bacteria) [42] of most antimicrobials for the major pathogens remained stationary when compared to those of the preceding VetPath surveys [43].

European Food Safety Authority (EFSA) [44] reported *S. aureus* and *E. coli* as being the most relevant antimicrobial-resistant bacteria in cattle in the EU. *Staphylococcus* spp. isolates have been reported to be resistant to β-lactams, tetracyclines, aminoglycosides, amphenicols, macrolides, trimethoprim, lipopeptides,

and lincosamides in Europe [45]. On the global level the highest mean levels of resistance in *S. aureus* were observed for penicillin, according to the data collected in the period from 2010 to 2021. The mean proportions of resistance in Oceania (23.9%) and Europe (32.1%) were substantially lower than in Africa (57.7%), South America (59.9%) and Asia (64.2%). In European countries, the highest penicillin resistance was reported in Italy (63.1%), whereas the low levels of resistance were observed in Sweden (4%), Austria (10%), (14%) and Denmark (17.5%). Methicillin resistance was less common in Oceania and South America (< 3%), when compared to Africa (8.8%), Europe (9.9%) and Asia (19.1%). Resistance to the third generation cephalosporins (3GCs) was less apparent, ranging from 0% for ceftiofur in Africa to 13.7% for cefoperazone in Europe. Although resistance to the lincosamide pirlimycin was generally low (< 5%), 47% of 100 isolates exhibited resistance in Austria. Mean fluoroquinolone resistance levels were higher in Asia (20.5%) than in other continents, including Europe (7.9%), except in Italy where 36.9% of 122 isolates were resistant to enrofloxacin. Resistance to macrolide erythromycin was highest in Asia (30.9%) and Oceania (28.8%), while in South America and Europe was estimated at 4.9 and 5.5%, respectively. Resistance to neomycin was generally low, with exceptions of Canada (18.3%; reported as the resistant and intermediate) and South Africa (16.7%; reported as intermediate). Very low levels of resistance were observed for sulfonamide–trimethoprim in most continents, including Europe (0.6%). The highest mean resistance proportion (37.9%) was detected in Asia. Data obtained for penicillin–novobiocin showed no or very little resistance suggesting this antimicrobial to be effective for the treatment of *S. aureus* mastitis [44]. Similar findings have been recently reported; the highest overall prevalence of resistant *S. aureus* was against penicillin followed by clindamycin, erythromycin and gentamycin, while ceftiofur and cephalotin had the lowest overall prevalence. However, the AMR to almost all the antimicrobials showed an increasing pattern over time, among which clindamycin, gentamycin, and oxacillin had a higher increase in their AMR prevalence [46]. Contrary to *S. aureus* mastitis that respond poorly to antimicrobial therapy [46], intramammary infections caused by CoNS are usually self-limiting, although some clinical mastitis cases require antimicrobial treatment [47].

A weighted mean proportion of 10.9% resistance in *E. coli* was reported for 3GCs [44]. Despite the detection of 43.3% of 102 isolates resistant to ceftiofur in Ukraine, less than 8% of *E. coli* isolates were found to be resistant to 3GCs in Europe. Only one study tested cefoperazone and reported low resistance of 0.8% among 135 isolates in France. Resistance levels for aminopenicillins were similar in Africa (44.9%) and Asia (40.1%) and higher than in Europe (31.1%). In addition, in France 34% of *E. coli* isolates were resistant to amoxicillin, while the highest resistance percentage of 77.45 was recorded in Ukraine. Ampicillin resistance ranged from 11.3% in Denmark and 12% in Germany to 39.4% in the UK. Mean resistance levels were lower for amoxicillin–clavulanic acid compared with ampicillin, with the highest levels detected in Chine (81% of 100 isolates). In Europe the resistance was estimated at 13.3%. Mean proportions of fluoroquinolone resistance were low, particularly in Europe (3%). Contrary, the mean resistance proportions of 22% were detected in Asia. Higher mean resistance percentages were observed for gentamicin (35.4%) and neomycin among isolates in Asia (11.8%) compared with Europe (20.6% and 9%), where the highest resistance was observed for gentamicin in Ukraine (26.5%). Lower average levels of resistance were observed for sulfonamide–trimethoprim (12.6%) and tetracyclines in Europe (22.4%) when compared to other continents [44]. Resistance to β-lactams, tetracyclines, and amynoglycosides appears to be widespread [45]. In comparison, higher levels of

resistance were observed in *E. coli* isolates from gastrointestinal cases than from mastitis cases for clinically important antimicrobials. Treatment of mild or moderate *E. coli* and other Gram-negative mastitis cases with antimicrobials is not warranted, while the use of antimicrobials to treat acute cases may be considered [30, 44].

Resistance levels were found to be similar for *S. uberis* and *S. dysgalactiae*. Overall mean levels of resistance for 3GC and penicillin were low and less than 7% in Europe. For the macrolides, most studies reported less than 25% resistance [44]. For lincosamide pirlimycin the resistance in *S. uberis* and *S. dysgalactiae* from Europe was 15.9% and 7.6%, respectively [43]. The mean proportion of sulfonamide–trimethoprim resistance for *S. uberis* ranged from 4.9% in North America and 12.7% in Oceania to 15.2% in Europe, while for *S. dysgalactiae* was 0.3% in North America, 7.4% in Europe, 14.3% in Asia, and 17.2% in Oceania. For fluoroquinolones, the mean proportion of resistance was 27.4% for *S. uberis* and 22.4% for *S. dysgalactiae* in Europe [44]. A recent review on AMR in bovine mastitis pathogens in European countries reported resistance of streptococci to macrolide, lincosamide, and streptogramin and the differences between *S. uberis* and *S. dysgalactiae* in the sense of a higher resistance prevalence in *S. dysgalactiae.* Generally low resistance to β-lactam antimicrobials was observed [45].

A few groups of antimicrobials are considered to be effective against mycoplasmas [48, 49]. The resistance in *M. bovis* to tetracyclines, macrolides, lincosamides, aminoglycosides, chloramphenicols, and fluoroquinolones appears to be rising [50]. High MIC values for spectinomycin, gentamycin and kanamycin were reported for isolates from milk [50]. Significant differences on the MIC values were found among Belgium, Germany and Italy for lincomycin, spiramycin, tylosin, oxytetracycline, florfenicol, enrofloxacin; however, a high level of resistance for macrolides and a low level of resistance for tiamulin and doxycycline were observed in all countries [51]. In China, the isolates had low MIC values to enrofloxacin and tiamulin [52]. Valnemulin was found to be effective against Spanish isolates [53]. Sensitivity of *M. bovis* to pirlimycin, danofloxacin and enrofloxacin, but not kanamycin, oxytetracycline, tilmicosin or tylosin was reported in Japan [54]. However, *M. bovis* mastitis is considered to be untreatable, and culling is the most common recommendation for its control [55].

Despite observed low resistance of major bovine mastitis pathogens to several cephalosporines and fluoroquinolones and the significance of these antimicrobials in veterinary medicine (categorised as VCIA or VHIA) [25], they meet the criteria related to human health: "A": High importance of the antimicrobial to human health to treat serious, life-threatening infections that have no or limited availability of alternative treatments and B: Risk of transmission of resistance to the antimicrobial from animals to humans, including cross-resistance or co-selection of resistance to other crucial antimicrobials [56]; "Critically Important" antimicrobials for human medicine, and thus should not be used as the first-line treatment in animals [57].

The AMR rates and patterns may vary by country or in one region over time, mainly depending on the (non)use of specific antimicrobials, bacterial species/strains and variable level of resistance among them, and the development and transmission of antimicrobial resistance. However, the data on AMR should be taken with precaution due to the limiting factors, such as the lack of information from many countries, the geographical and temporal variations, variable number of tested isolates being collected prior to antimicrobial treatment or after, the variety of available antimicrobials, methodologies (disk diffusion, broth microdilution, agar dilution), interpretative criteria (clinical breakpoints/epidemiological cut-off values) and the differences related to the Clinical Laboratory Standard Institute (CLSI) [58] and the European

Committee on Antimicrobial Susceptibility Testing (EUCAST) [59] guidelines [44, 46]. Because of the differences between CLSI and EUCAST recommended disk contents and commercial availability in the countries, these organisations initiated common criteria for development of optimal disk contents (potencies) in 2017 [60]. CLSI [58] is the only organisation providing internationally available methods and breakpoints specifically for many bacteria from animals [41, 61]. The results of AST provide guidance of potentially suitable antimicrobials; however, harmonised AST methods, veterinary-specific interpretive criteria are not available for all antimicrobials, bacterial pathogens, animal species and sites of infection, including those for bovine mastitis pathogens [41, 61]. The correct evaluation of AST results requires veterinary-specific clinical breakpoints (VSCBs) and quality control ranges [61]. Thus, the accurate status of AMR among mastitis-causing bacteria is largely unknown and the data so far reported are uncertain. These drawbacks underscore the urgent need for standardised guidelines for the AST and interpretation criteria, as prerequisites for adequate therapy, AMR monitoring and reporting at national and regional levels, and the harmonisation of a global AMR surveillance system.

3. Treatment of bovine mastitis: success or failure?

The outcome of mastitis treatment depends on many factors, such as the resistance of the causative pathogen against the chosen antimicrobial agent [61] and the lack of correlation between antimicrobial *in vitro* activity and *in vivo* efficacy [16]. Therapy with antimicrobials to which bacterial isolates showed susceptibility *in vitro* results in a low proportion of cure *in vivo*, and conversely bacterial isolates that are resistant to antimicrobials *in vitro* may cure following treatment *in vivo* [16]. This antimicrobial *in vitro*/*in vivo* paradox is difficult to explain, mainly because of unexplored host-pathogen–antimicrobial interactions and resulting responses/effects. However, special consideration should be given to several factors. Insufficient improvement of clinical signs might be related to specific physicochemical conditions at the site of infection (e.g., pH value, oxygen partial pressure and perfusion rate) [61]. Appropriate choice of antimicrobials, the pharmacodynamic and pharmacokinetics properties of antimicrobial agents, drug interactions, the selection of optimal antimicrobial drug regimens: dosing, duration of therapy, routes of administration and optimal therapeutic concentrations should be carefully addressed [30, 61].

The most common *in vitro* AST methods are disk diffusion, broth (micro) dilution and agar dilution [59, 62]. Based on the breakpoints the bacterial isolates are categorised as "S" "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent; "I" "Susceptible, Increased exposure" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection; "R" "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure [59]. According to CLSI "I" still stands for intermediate and "SDD" is a separate category "Susceptible Dose-Dependent". Clinical breakpoints are defined according to *in vitro* and in *vivo* data to predict the likelihood of clinical cure [30]. Thus, the determination relies on the distribution of MICs within the target bacteria species, combined with pharmacokinetic-pharmacodynamic parameters and data from clinical efficacy studies [30, 61]. These data are still unavailable for various antimicrobials for bovine mastitis pathogens [41]. Analysis for many antimicrobials specific to animals, including cattle

and disease depends on breakpoints based on data specific for humans (MIC data, pharmacokinetics, particularly the serum concentrations, and clinical outcome of human patients) [41]. Currently, several antimicrobials have interpretive guidelines for bovine mastitis pathogens, and categorisation of susceptibility and resistance still relies on clinical breakpoints developed for humans [41]. In view of clinical efficacy, incorrect data on AST can be misleading for the choice of antimicrobial drugs resulting with inadequate therapy and AMR [61]. In addition, standardised procedures for MIC testing of antimicrobials against veterinary mycoplasmas (including *M. bovis*, one of the major bovine mastitis pathogens) and criteria for interpretation are lacking, while standard procedures such as the disk diffusion method are not recommended for mycoplasmas due their fastidious nature [49, 63]. There is the lack of ECOFFs/ECVs (i.e., the highest MIC for organisms devoid of phenotypically detectable, acquired resistance mechanisms, which defines the upper end of the wild-type MIC distribution), a necessary step when setting clinical breakpoints to guide therapy. This also prevents the separation of isolates with (non-wild-type) and without (wild-type) phenotypically detectable resistance and affects AMR surveillance and early warning of developing resistance [62, 64]. Nevertheless, ECOFFs are not adequate for classification of isolates as clinically resistant or to calculate the percentage of isolates that are multidrug-resistant (MDR) (defined as an isolate that is not susceptible to at least one agent in at least three antimicrobial classes) or extensively drug resistant (XDR) (defined as an isolate that is not susceptible to at least one agent in all but one or two antimicrobial classes) [65, 66] due to the lack of relevant pharmacological data [30].

Antimicrobial resistance occurs when bacteria have or develop to avoid the mechanisms of the drugs against them, the ability to replicate and not just survive in the presence of a drug [42, 67]. The most common measure of the level of resistance is MIC and a higher MIC corresponds with a higher level of resistance [42]. Natural resistance may be intrinsic, which is always expressed in the species, and induced, when naturally occurring genes are only expressed to resistance levels after exposure to an antimicrobial [68]. Acquired resistance is exhibited when a previously sensitive bacterium acquires a resistance mechanism [67] by mutations in chromosomal genes or acquisition of the genetic material from an exogenous source by horizontal gene transfer (HGT) that can occur through transformation, transduction, and conjugation [18, 67, 68]. Sub-MIC antimicrobial concentrations can positively select for resistance mutations, increase HGT of antimicrobial resistance genes (ARGs) and mutation rates [69]. The inoculum effect (higher initial density of cells resulting in lower susceptibility to some antimicrobials) may lead to the failure for treating infections because the actual MICs of bacterial populations are higher than those determined *in vitro* (e.g., bacteria producing antimicrobial-inactivating enzymes, higher rates of degradation correlate with higher number of bacterial cells) [69, 70]. Switch from resistance to susceptibility is not common [70]; bacteria are able to survive antimicrobials without encoding specific resistance mechanisms [69].

The contribution of non-inherited, phenotypic resistance to antimicrobial treatment failure appears to be significant [71]. Drug indifference occurs when the antimicrobial is effective only in a specific bacterial physiological condition (e.g., non-dividing cells are resistant to some antimicrobials, whereas other antimicrobials are active against stationary cells, but their level of activity is lower than when cells are actively growing). The antimicrobial concentrations required for curing an infection are directly related to the duration of the infection [70, 71]. In addition, the phenomenon known as "bacterial persistence", "adaptive resistance" and "phenotypic

tolerance" [71] may be responsible for the differences between the *in vitro* and *in vivo* effectiveness of an antimicrobial and involved in the clinical failure of antimicrobial treatments. It describes transient resistance to one or more antimicrobials, induced by a specific environmental signal (e.g., stress, subinhibitory levels of antimicrobials) or due to epigenetic phenomena like persistence that allows bacteria to respond more rapidly to antimicrobials [67, 72]. The increase in resistance as a response to environmental changes may not completely revert upon removal of the stimulus. This can lead to a gradual increase in MIC over time [67]. Both persistence and tolerance describe increased survival in the presence of an antimicrobial without an increase in the MIC [42] and allows bacteria to resume normal growth once the antimicrobial is removed [70]. While resistance and tolerance are considered properties of a population, persistence refers to the ability of a subset of the population to enter a state of dormancy and survive exposure to high concentrations of antimicrobial, whereas the rest of the population is rapidly killed [42, 70]. Therefore, persisters (persistent cells or a subpopulation of tolerant bacteria) [42] are predominantly dormant and can survive courses of antimicrobials, since antimicrobials are most effective against actively-metabolising cells. Moreover, they are also relevant in biofilms [73]. The level of persistence (the size of the persister subpopulation) will only weakly depend on the concentration of the drug if it is far above the MIC. The survival advantage of persisters is often observed for antimicrobial treatments belonging to different classes of antimicrobials [42]. Poor therapy response can also be explained by the lack of microbiological testing, inappropriate diagnosis [61] and polymicrobial infections [70]. Undetected mixed/polybacterial or polymicrobial infections and mastitis pathogens missed by standard culture pose a high-risk for treatment failure, the occurrence of recurrent infections, reservoirs of infection and dissemination of pathogen (s) among the cows. Culture-negative milk samples have been frequently observed from cases of clinical mastitis (40% of samples) [16], whereas, for example mycoplasmas have been rarely investigated in undiagnosed cases of mastitis (over a quarter of clinical and nearly 40% of subclinical cases) [55, 74]. Considering that multiple bacterial and/or other pathogens may be involved, such as fungi and algae, or mycoplasmas undetectable by conventional methods, antimicrobial therapy most likely was ineffective if microorganisms isolated from a mastitis sample are not primary pathogens [30]. Moreover, clinical susceptibility may not provide the probability of treatment success of polymicrobial infections where pathogens are embedded in complex multispecies microbial communities due to intra- and interspecies interactions that alter species responses under antimicrobial exposure [70]. Because resistance is determined by the interactions within that specific community AST should be conducted upon communities in addition to single-cell cultures [70]. Survival strategies of bacterial communities in the presence of antimicrobials are: (1) Collective resistance, interactions within a community that elevate the ability of its members to resist the action of an antimicrobial and continue to grow in the presence of antimicrobials thus increasing the MIC of the community; (2) Collective tolerance, interactions within a community that alter cell state, such as slowing down metabolism, and thus slow down the rate of cell death during transient exposure to antimicrobials without an increase in MIC; (3) Exposure protection, interactions within a community that protect its sensitive members during antimicrobial treatment by reducing the effective concentration of antimicrobial. These three main modes can additionally be enhanced by biofilm formation [70].

A biofilm is often defined as "an aggregate of microbial cells adherent to a living or non-living surface, embedded within a matrix of extracellular polymeric substances (EPS) of microbial origin". EPS is combined of extracellular macromolecules

including nucleic acids, proteins, polysaccharides and lipids. Clinical biofilm-associated infections should be distinguished from microbial colonisation with non-pathogenic organisms [75]. Microorganisms (single or multiple microbial species) initiate biofilm formation under environmental pressure, such as antimicrobial treatment and subinhibitory concentrations of antimicrobials [70, 76]. Biofilms serve as barriers against host immune responses and drugs and protect their members through limiting the diffusion of antimicrobials into the population and increasing the protection provided by antimicrobial inactivation [69, 76]. This leads to resistance to antimicrobial treatment and reduction the possibility of eradicating infections [76]. Biofilms can also increase the proportion of persister cells within the population and levels of resistance by altering the expression of pre-existing ARGs [70]. One of the reasons for difficulties in resolving chronic mastitis cases is biofilm formation. However, most of the studies on biofilm associated with bovine mastitis are *in vitro*, living a gap on the composition, mechanisms of biofilm development, interactions between the host and biofilms, and the factors that can affect outcomes (increased or decreased biofilm formation) not necessarily linked to the use of antimicrobials [77]. Many bovine mastitis pathogens are able to produce biofilm, including *S. aureus,* CoNS, *E. coli*, *S. agalactiae*, *S. dysgalactiae*. *S. uberis, E. faecalis* [78], mycoplasmas [79], *Candida* spp. [80], and *Prototheca* algae [81]. The high resistance of biofilms to current antimicrobials makes its eradication very difficult; nevertheless, there are new promising strategies like antimicrobial peptides, nanotechnology, ozone, bacteriophage therapy, apitherapy and phytotherapy [27, 77].

Other factors involved in treatment success or failure include the lack of microbiological testing, AST and the evaluation of antimicrobial therapy. Microbiological testing of milk samples and AST of the isolates should be performed prior to therapy, in a prompt and timely manner. Repeated microbiological testing, approximately 7 days (depending on the used antimicrobial) following course of antimicrobial therapy is necessary to ensure clearance of infection and to exclude carriage. The postponed clinical responses should be avoided, as the delayed onset of improvement should not be interpreted as treatment failure [61]. Spontaneous cure (in the absence of antimicrobial treatment) of intramammary infections is recognised in dairy cattle, and thus antimicrobial treatment is not always required for resolution of clinical signs or bacteriological cure of intramammary infections [16].

4. Mastitis control programmes

Preventive measures based on cow health control, biosecurity and farm management are essential for effective mastitis control [82, 83]. Improving udder health at farm level is based on the reduction in duration of existing intramammary infection and reducing the incidence of new intramammary infection [4]. The “five-point plan” in the UK (routine maintenance of milking machines, post-milking teat disinfection, identification and antimicrobial treatment of clinical cases, whole herd antimicrobial dry-cow therapy and the culling of chronically infected cows) has been very effective in managing contagious pathogens until the rise in environmental pathogens and, therefore a need for some adaptations [32]. Following the ten-point mastitis control programme by National Mastitis Council (NMC) of USA was based on ten steps: 1. establishment of goals for udder health, 2. maintenance of a clean, dry, comfortable environment, 3. proper milking procedures, 4. proper maintenance and use of milking equipment, 5. good record keeping, 6. appropriate management of

clinical mastitis during lactation, 7. effective dry-cow management, 8. maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows, 9. regular monitoring of udder health status, 10. periodic review of mastitis control programme [84]. Mastitis control programmes and their effectiveness vary by country, and in some states, such as Norway and the Netherlands [85, 86] appear to be very successful. Unfortunately, such programmes lack in many parts of the world. Regardless of the control strategy, mastitis control programmes include antimicrobial therapy [16] and knowing the mastitis pathogens is critical to the rational use of antimicrobials [30]. Culturing of mastitis cases can dramatically reduce the number of cows that are treated with an antimicrobial [30]. For detection in a timely manner and to avoid false-negative results, milk samples from individual cowsand pooled with the number of milk samples lower than those in bulk tank milk should be examined for pathogens on a regular basis and sequentially, using culture-based methods combined with real time PCR or other highly sensitive molecular technique. The preventive and control measures should also include enhanced biosecurity on farms, regular controls of animal/human movement, quarantine and testing of purchased cows prior to introduction to farms, separation of suspected and removal of infected cows, proper milking and environmental/housing hygiene, correct dry-cow management, nutrition and vaccination and other actions contributing to improvement of cow health, immunity and welfare. Increasing farmer awareness of mastitis control strategies and AMR and communication with veterinarians are also crucial in combating and preventing cattle mastitis and reducing of overuse and misuse of antimicrobials. Efforts toward effective control and prevention of mastitis and prudent use of antimicrobials reflect in research on the development of new vaccines and alternatives to antimicrobials such as the use of bacteriophages, nanoparticles, cytokines, animal- and plant-derived antimicrobialcompounds, antimicrobial proteins, probiotics and prebiotics and homoeopathy [26, 27, 30].

5. Conventional therapy/prevention and alternatives to antimicrobial therapy

The emergence of resistant bacteria related to the treatment effectiveness, public health risks and the environment have raised the need for the novel therapeutic approaches [27]. However, the main course of treatment of bovine mastitis still relies on antimicrobial use. Antimicrobials are most often administered either by intramammary route or the systemic route [27].

After the advents of the antimicrobial era that produced an effective intramammary treatments, antimicrobial usage in dairy cows usually occurs as [87, 88]:

Primarily, clinical mastitis is mostly treated by intramammary administration of antimicrobial formulations (local treatment). Severe mastitis requires additional antimicrobials administered parenterally. Secondly, local antimicrobial treatment is performed at the day of drying-off, 45–60 days before the next calving. Drying-off treatment has shown significant effect in the reduction of mastitis and has enabled many dairy farms to reduce or even eliminate specific pathogens from their herds (dry-cow treatment – DCT). Usually, this procedure has been recommended for all cows at dry-off worldwide. Quite often when antimicrobial treatment is done (intramammary or parenteral) during any time of lactation period, milk is not suitable to be used and has to be discarded because of drug residues. After antimicrobial treatment is finished, withdrawal period has to elapse whose duration depends on

pharmacological properties of used drug and during that time farmers experience economic losses. Beside main antimicrobial therapy, in more severe cases additional symptomatic and supportive therapy are of crucial importance to reduce local inflammatory process in the affected quarters, to enable a better perfusion of antimicrobial through tissues, as well as more rapid mammary tissue healing and restoring milk production. Regarding the current discussion about AMR, the described blanket antimicrobial DCT seems obsolete, although no data confirm that DCT bears relation to the emergence of AMR of mastitis or human pathogens [88]. Unfortunately, common use of antimicrobial therapy has made it undesirable in many aspects considering public health. Common utilisation of antimicrobials intentional or not may leave the significant residues in ecosystems and food chain and lead to development and spreading of resistant microorganisms. In the industry of fermented milk products these residues may cause a serious and even disastrous problem affecting all lines of production and health of final consumers.

Currently, according to reports approximately 30,000 humans in EU and 700,000 humans globally die every year from infections caused with multiresistant bacteria. Without solutions leading to a reduction in AMR, since 2050 approximately 10 million people annually are in great death risks from bacterial infections caused by bacteria with AMR [88]. Moreover, through cows and their products possibility of creating new resistant strains always exist and that fact have affected public concern, emphasising importance for the reduction of antimicrobial usage in food-producing animals.

Despite the use of antimicrobial dry-cow therapy, influence of pathogens from the environment to the appearance of intramammary infections or clinical mastitis is still quite common [88]. Formation of keratin plug in teat canal during dry period could be delayed or insufficient, which is a great risk factor for development of new intramammary infections. Mammary quarters with open and/or damaged teat canal have almost double risk to develop new intramammary infection during the dry period, compared to closed or undamaged teat canal. Using combination of antimicrobial dry-cow therapy and internal teat sealant to mimic the protective effects of the keratin plug and provide protection during the entire dry period, provides benefits over antimicrobials alone through improved prevention of new intramammary infections, subclinical mastitis, reduced somatic cell count and reduced use of intramammary antimicrobials in next lactation [89, 90].

The discovery and development of new treatment agents as alternatives in bovine mastitis therapy, comes together with consumers demand for antimicrobial-free products, which has led to several new options in therapy and prevention.

6. Vaccination

Considering historic and modern importance of vaccination in almost all areas of animal breeding, it is logical choice to formulate and implement certain vaccinal programmes in bovine mastitis prevention. In veterinary medicine vaccination programme is important and effective method in prevention and control of many infective diseases. However, unfortunately just several vaccines proved to be effective in routine practice. True success for any vaccinal regime depends on quality of vaccine, route of administration as well as coverage of vaccination among cows. Reports indicate that results in vaccine efficacy are quite different and to obtain satisfactory results many control measures have to be implemented as part of mastitis control strategy, because vaccination alone will not be solution on its own [27].

6.1 Nanotechnology

This relatively new technology often called nanotech has become a growing methodology in the 21st century with a great potential to be used as comprehensive tool in various industries. Nanoparticles offers many new and different types of materials to be used in veterinary medicine (nanotherapy), as well as reducing the problem of AMR and drug residues. Nanotherapy is making a significant economic influence in dairy industry, reducing the quantities of discarded milk and culled cows from herds. New delivery systems created by nanoparticles enables antimicrobial drugs to be used efficiently in low dosages directly into the target cells with shorter withdrawal period, leading to the reduction of side effects and financial losses. Nanoparticles are able to perform higher intracellular drug uptake compared to other typical ways of drug delivery systems. In this manner accumulation, antimicrobial activity and the retention time of the drug is increased, AMR is decreased and finally biofilm formation is inhibited [91]. To establish better control and overcome therapeutic difficulties against *S. aureus* related mastitis, inorganic nanoparticles like nanogels and antimicrobials have proven to act synergically and highly effective [91, 92].

6.2 Probiotics

According to numerous studies, probiotics have great potential for improving health and well-being. Classification of probiotics as probiotic drug mean that probiotic is associated with a certain medical condition and can be used as therapy or to prevent disorders. Lactic acid bacteria originating from the teat canal microbiome could be used in mastitis prevention. Live culture of *Lactococcus lactis* after intramammary administration proved in some cases to be effective like antimicrobial treatment, but without any withdrawal period. The infusion of *L. lactis* into the bovine mammary gland promoted recruitment of neutrophils, and increased concentrations of milk acute-phase proteins and expression of genes encoding cytokines IL-8 and IL-1 β. Isolates of the *Lactobacillus* and *Lactococcus* genera showed inhibitory activity toward some major mastitis pathogens like *S. aureus*, *S. uberis* and *E. coli* [93].

6.3 Phytotherapy

Utilisation of plants is part of traditional medicine worldwide and is one of the most promising alternative options in the prevention and treatment of health disorders. Many traditionally used medical plants possess antimicrobial, anti-inflammatory, antioxidant and immunomodulatory potential. Biological diversity of herbs from numerous world regions provides an almost endless choice of raw materials with huge potential in medicine. Possibility of being used synergically with antimicrobials, highlights the importance of medical plant–antimicrobial combinations against common pathogens, as well as against resistance-modifying agents. Some essential oils extracted from plants express even antibiofilm properties. Plant extracts may be utilised in different ways like infusion, gel, spray or ointment. Some researchers reported that the effect of phytotherapeutical remedies used in mastitis treatment was similar to conventional antimicrobial therapy but without an irritating effect on the udder and had minimal residues in milk. One of the promising alternative phytotherapeutics against mastitis pathogens is Cinnamon essential oil. It shows powerful bactericidal characteristics with beneficial anti-inflammatory effects

and a reduction in tissue damage in mammary gland. Essential oils have also shown strong antimicrobial activity against causative agents of protothecal mastitis, as well as against some other typical mastitis pathogens like *Staphylococcus sp*., *Streptococcus* sp., *Bacillus cereus* and *E. coli*. Unfortunately, undesirable properties like instability, biodegradability and low solubility of essential oils in certain solutions exist but could be improved in combinations with nanomaterials to improve their transport and efficiency. Phytotherapy utilisation may highlight significant economic benefits, especially with a focus on subclinical mastitis, because it is responsible for most of the financial losses [27, 92, 94] and could have a great potential to be used not just in conventional but especially in organic farms.

6.4 Bacteriophages

They are defined as viruses with ability to infect bacteria and to continue replication inside of them, suppressing their proliferation. Their important ability is to be able to target specifically only the pathogens of interest, while microbiome of the host is not affected. The main limitation of the phages is their specificity. Single bacteriophage can affect only a certain number of bacterial strains, and treatment of infection caused by several possible bacteria requires different phages. To increase potency, administration of phages can be in the form of cocktails or together with some antimicrobials. This makes them desirable for treatment against multidrug-resistant bacteria, possessing low probability of resistance development. Moreover, phages are degraded in nature after solving infection, while antimicrobials can persist for a long time. Phages are a powerful option for the post-antimicrobial era, especially against drug-resistant bacteria, where they also reduce the number of somatic cells, contribute to inflammatory factors, relieve the signs of mastitis in cattle and even potentially be used in the development of vaccines [95].

6.5 Low intensity laser radiation

In recent years this methodology represents an alternative and non-pharmacological therapeutical way with many previous positive uses in humane medicine. To perform treatment, every udder quarter have to be irradiated, divided in daily treatments and cycles. Beneficial response from the cows is expressed in the form of decreased number of microorganisms, more receptive microorganisms to antimicrobials treatment or blood vessel regeneration. Laser irradiation stimulates the phagocytic activity of milk granulocytes, becoming more active in destroying the etiological agents of mastitis. Irradiation treatment increases healing rate from mastitis treated intramammarily or intramuscularly with antimicrobials. Compared to antimicrobial treatment alone, irradiation enables faster regression of clinical signs such as redness, pain, hardening, inflammation, and oedema, promotes healing of wounds, deeper tissues and nerves and prevents cell death and tissue damage, as well as faster disappearance of macroscopic changes in milk and better elimination of intramammary infections. The supportive effect in treated mammary glands is probably due to the regulatory effect on pro- and anti-inflammatory cytokines *in vivo* and *in vitro* and with stimulation of the immunological system in vivo. Temporarily, the higher number of somatic cells in treated cows may be due to a bio-stimulating effect because, at the tissue level, laser therapy stimulates the immune system by accelerating phagocytosis, blood and lymph circulation and intracellular generation of active oxygen forms. Effectiveness of treatment increases when repeated for several days [96–98].

6.6 Ozone

Ozone as a gas represents polimerised oxygen (O_3), created by ozone generator or under the influence of ultraviolet light. Application of ozone in desinfection and reduction of microbial population and decontamination is well documented. The bactericidal, fungicidal and virucidal properties of ozone, through a strong oxidation effect, seem to have significant potential in the treatment of mastitis in bovines [99]. Local and systemic signs tend to improve after ozone administration to clinically inflamed quarters. Compared to antimicrobial administration, milk is not discarded during treatment or after. Chronical mastitis proved to be more difficult to cure totally by ozone administration alone, and certain microbials like *S. uberis* shows good resistance against ozone treatment. In these situations, ozone therapy should be combined with antimicrobial treatment. In general, ozone treatment is cheap and with similar effectiveness as antimicrobial treatment for clinical or chronical mastitis, but also reduction in somatic cell number seems to be somehow faster [99, 100]. Other reports coincide with statement that ozone administration in cases of clinical and subclinical mastitis in dairy cattle may lead to elimination of causative agents and detoxification of the inflamed quarter [101, 102]. Even ozone water seems more effective when compared to antimicrobial administration, especially in cases of coliform mastitis when irrigation of a quarter is needed. Irrigation with ozone water may cause lower endotoxin release from *E. coli* to the milk other than the treatment with local or systemic antimicrobials [101]. Minor problems could appear in routine practice with ozone treatment because it usually requires several cycles of treatments [100–102].

6.7 Apitherapy

Apitherapy has been used as a traditional remedy since ancient times for possessing various therapeutic activities (antimicrobial, anti-inflammatory, antioxidant, antiproliferative, immunomodulator). This method is very safe, highly effective, easily applicable and extremely economic. Apitherapeutic management is gaining more importance in the modern medicine and could be used for many varieties of different health disorders. This is especially important in food-producing animals as it is highly safe for cow products and very effective. Apiproducts like honey, pollen, propolis and venom, proved to have wide effectiveness, which depends on botanical, geographical and seasonal conditions, leading to differences in their potency. Multiple compounds contained in bee products act synergistically and are very effective in different concentrations against even multi-drug-resistant bacteria, besides boosting immunity and antioxidative effect. Apiproducts act as natural compounds with none or minimal irritation to tissue, even to sensitive mammary tissue, which is very susceptible to irritation. Administration of honey intramammary has beneficial effects against bacteria, but it also led to an increased number and activity of total leucocytes, helping to resolve mastitis and eliminate causative agents [103]. Diluted or even undiluted honey may be used as intramammary treatment against bacteria like *S. aureus*, *P. aeruginosa* and *E. coli*, while it is harmless to mammary tissues and without undesirable residues in milk. Because repeated administration does not produce any microbial resistance, it could be a good choice for mastitis treatment in conventional and organic farms [104–106]. Besides honey, propolis is also one of the well-known and used honeybee products. This substance has a complex chemical composition with many expressed biological activities. Intramammary administration of propolis showed significant antimicrobial, antioxidant, immunostimulatory and anti-inflammatory abilities.

These activities in mammary gland makes propolis great alternative to conventional therapy, and some reports proved it is even more effective against Gram-positive bacteria than Gram-negative bacteria [107–109]. Propolis have ability to even reduce the growth of typical mastitis pathogen like *S. aureus* to an average of zero [107], but to reduce reaction of mammary tissue concentration must be lower [108]. Surprisingly, even honeybee venom usually utilised as pain reliver and in treatment of inflammatory diseases is highly effective against typical mastitis pathogens including methicillin-resistant *S. aureus* (MRSA) without side effects even in its lowest concentrations [110, 111].

6.8 Homoeopathy

In the last decade, this method is gaining popularity for food-producing animals, especially in organic farms. Methodology is based on a holistic approach with the goal of stimulating the cows' immune system and fighting against AMR. In India, therapies with homoeopathic remedies and their combinations have proved effective against mastitis. Even their combination with certain antimicrobials could be part of the solution in the successful control of bovine mastitis. Side effects or particular allergy reactions are not common, and there are no residue problems or withdrawal period in milk or the product, non-environmental pollution and these remedies are for many farmers. However, references to homoeopathy are still relatively limited and more research in the area of holistic remedies and treatments is required to prove real medical efficiency of this approach [112–114].

6.9 Bacteria-derived antimicrobials

This group of antimicrobial peptides is active against many Gram-positive and Gram-negative bacteria. Compared to antimicrobials, these peptides like bacteriocins have a very narrow spectrum of antimicrobial activity, which allows them to target only specific pathogens and work efficiently even against antimicrobial-resistant strains. Bacteriocin nisin produced by lactic acid bacteria (*L. lactis*) proved to have an inhibitory effect against mastitis-related pathogens like *S. uberis* and *S. agalactiae*. These peptides are becoming desirable therapeutic options in food-producing animals for activities against mastitis-related and foodborne pathogens. There are some new reports about successful mastitis treatment with bactofencin, nisin and reuterin. All of them were highly active against multidrug-resistant mastitis isolates, while nisin could even express antimicrobial activity on biofilm-producing *S. aureus* cultures. Certain bacteriocins can act synergistically with conventional antimicrobials, leading to reduced drug concentrations, decreased side effects, and the appearance of new resistant strains [115–118].

7. Animal-derived antimicrobials

Milk contains peptide substances like lactoferrin and other similar proteins with antimicrobial properties such as immunoglobulins, lysozyme, β-defensin and lactoperoxidase. These peptides have a broad spectrum of activities, which control many biochemical processes. They all can potentially be used in treating various infectious diseases caused by bacteria, fungi and protozoa. Their activity can neutralise toxins, inactivate bacteria, and limit or prevent bacterial adherence to the mammary tissues.

The property of lysozyme to hydrolyse the essential bacterial cell component peptidoglycan, was used successfully in increasing antimicrobial efficacy against *S. uberis* and *S. dysgalactiae*. Spectrum of antimicrobial activity may even increase in combinations like lactoferrin and β-lactoglobulin, because of their different activities on different bacteria. More than 60 antimicrobial peptide drugs have already reached the market, while more therapeutical peptides are yet to come, waiting to finish preclinical and clinical development [119]. All above-mentioned peptides could be considered as possible non-antimicrobial agents against bovine mastitis-related pathogens with further potential to be used with antimicrobials [119–122].

8. Conclusions

The use of antimicrobials remains the major approach for mastitis treatment. The standardisation of AST, determination of clinical breakpoints and interpretive guidelines for bovine mastitis pathogens are crucial for the appropriate selection and use of antimicrobials, AMR monitoring at national and regional levels, and the harmonisation of a global AMR surveillance system. The lack of routine microbiological testing of milk samples and AST of the isolates may lead to improper therapy, the persistence of mastitis, increased transmission of pathogens and AMR rise. Multiple mastitis pathogens missed by standard culture and biofilms are high-risk factors for treatment failure. The success of mastitis treatment highly depends on appropriate choice of antimicrobials, the pharmacodynamic and pharmacokinetics properties of antimicrobial agents, drug interactions and the selection of optimal antimicrobial drug regimens. In addition, AST of the isolates prior to therapy and the assessment of antimicrobial therapy are among the main steps for AMR monitoring and prevention of its occurrence. Implementing national mastitis control programmes and evaluation their effectiveness are imperative. Some countries are ahead of others in terms of improved approaches to mastitis management and control of antimicrobial consumption on dairy farms; their experience can guide the development of further strategies. Cow health control, udder health monitoring, improving farm management practices, identification and reducing the risk factors of mastitis, pathogen introduction and spreading, monitoring and restricting antimicrobial usage (and reserving the antimicrobial agents for the therapy), should be regularly applied until the development and implementation of more effective control measures, alternative farming systems and/or the decrease in consumption of cattle products.

Conflict of interest

The authors declare no conflict of interest.

Author details

Zinka Maksimović[1*], Benjamin Čengić[2], Amel Ćutuk[2] and Alan Maksimović[2]

1 Department of Pathobiology and Epidemiology, Veterinary Faculty, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

2 Department of Clinical Sciences, Veterinary Faculty, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

*Address all correspondence to: zinka.maksimovic@vfs.unsa.ba

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