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Blunt Antibiotic Weapons Against Mastitis

AUTHORS DETAIL

Tayyaba Akhtar[1]*, Muhammad Ifham Naeem[1], Muhammad Younus[2], Qamar un Nisa[3], Qaiser Akram[2], Hafiz Manzoor Ahmad[4], Waqas Ahmad[4], Shamreza Aziz[1] and Tayyaba Ameer[1]

[1]KBCMA College of Veterinary and Animal Sciences, Narowal, Sub-campus UVAS Lahore, Pakistan.
[2]Department of Pathobiology, KBCMA College of Veterinary and Animal Sciences, Narowal, Sub-campus UVAS Lahore, Pakistan.
[3]Department of Pathology, University of Veterinary and Animal Sciences, Lahore.
[4]Department of Clinical Sciences, KBCMA College of Veterinary and Animal Sciences, Narowal, Sub-campus UVAS Lahore, Pakistan.
*Corresponding author:
tayyabaakhtarcheema@gmail.com

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INTRODUCTION

Mastitis or simply put as the inflammation of mammary glands, is a global challenge faced by dairy farmers that results in major economic losses for the dairy industry (Hogveen et al. 2011; Gomes and Henriques 2016; Jamali et al. 2018). Mainly, these losses are attributed to reduced milk quality, lower milk yield, and increased use of drugs, particularly antibiotics that is further linked to some major problems like the antibiotic resistance phenomenon (Miller et al. 1993; Azooz et al. 2020). Some other economic losses as a consequence of mastitis include the increased expense of veterinary services, increased culling rate, and sometimes even death of the animals (Kumar et al. 2010). On an average, a farmer loses around $147 per cow per year due to mastitis (Hogeveen et al. 2019). Mastitis is a term used to indicate inflammation of the udder in mammals. More accurately the inflammation of udder parenchyma is referred as mastitis. It is characterized by usual inflammatory signs seen on the udder. These signs can include edema, swelling, increased temperature of the udder and abnormal physical and chemical characteristics of milk being produced by the affected udder (Radostitis et al. 2006; Paramasivam et al. 2023). Mastitis is classified according to the appearance of clinical signs as clinical or subclinical (Antanaitis et al. 2022). Furthermore, clinical mastitis can be acute, sub-acute, or per-acute depending on the severity of clinical signs (Kibebew 2017). The subclinical status of mastitis is the main hurdle in its control as it shows no visible signs (Abebe et al. 2016). Although, several efforts like the “Five-point-plan” and antibiotic therapy have been put into practice to control mastitis (Hossain et al. 2017; Breen 2019). However, recently the misuse of antibiotics in animals has led to the development of antibiotic resistance in pathogens of mastitis making it even more difficult to cure (Gomes and Henriques 2016). The rise of antibiotic resistance hence needed a remedy in the form of some other alternatives besides conventional antibiotics and the best of these turned out to be plant extracts (Zaatout 2022). An important reason for using plant extracts was their easy availability and affordability (Rakholiya et al. 2013; Yusuf et al. 2022). This chapter aims to explain antibiotic resistance in some important pathogens of mastitis along with its causes, effects, and treatment.

Antibiotic Resistance

Antibiotic resistance is a hot topic of several health security debates among concerned authorities of various countries. Antibiotics are our only chance against pathogenic bacteria and in recent years, widespread misuse of these drugs has also played a role in making our only weapon of defense, blunt against enemies (Oliver and Murinda 2012). The only way to undo our outrageous mistake is to closely monitor the surveillance of healthcare policies and practices by policymakers, researchers, and prescribers to mitigate the complex task of antibiotic resistance in the new era of science (Velez and Sloand 2016). Antibiotic resistance in microbial germs is a complex provocation worldwide. This villain isn't just another healthcare challenge of science but a baddie that comes with scoundrels that go by names of "high morbidity" and "high mortality" (Akova 2016). Antimicrobial resistance is also a matter of grave concern for health professionals and veterinarians alike because it is suspected to be transferable from animals to humans through the food chain. This is the focus of modern research related to antimicrobial resistance (Piddock et al. 1996).

Antibiotic Resistance against Mastitis

Beta-lactams have been used for a long time for the treatment of mastitis worldwide. Recently, there has been a sharp decline in the efficiency of beta-lactams due to the synthesis of beta-lactamase enzymes by bacteria to resist the effect of these drugs. Beta-lactamase is encoded in the bacterial genome by blaZ (Olsen et al. 2006). Some bacteria also have

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a mecA genome that codes for low-affinity penicillin-binding proteins (PBP2a). These proteins mediate methicillin or oxacillin resistance mechanisms to nullify the effectiveness of beta-lactams (Sawant et al. 2009). Bacterial pathogens also have natural defense systems against antimicrobial drugs. An example is the leading causative agent of mastitis is *Staphylococcus (S.) aureus*. It has a lower cure rate as compared to other mastitis agents. This lower cure rate is supposed to be because of the biofilm-forming abilities of this bacteria (Taponen and Pyörälä 2009). Biofilm-forming ability reduces the susceptibility of *S. aureus* against various antimicrobial drugs by reducing the diffusing efficiency of these drugs inside the biofilm matrix. Besides, providing *S. aureus* with antimicrobial resistance this biofilm also grants it enhanced bacterial adhesion ability to colonize mammary cells more easily and helps it evade harsh conditions and attempts of phagocytosis by the host's immune cells. Biofilm also increases the persistence of infections caused by *S. aureus.* There is an ica locus present in the genomes of *S. aureus* and *S. epidermidis.* This locus encodes icaA and icaD genes that are majorly responsible for biofilm formation (Arciola et al. 2001). Antimicrobial resistance is also a public health hazard for humans along with animals. It is responsible for low cure rates against bacterial diseases despite the use of antimicrobial drugs. Several strains of *S. aureus* recently collected from mastitis-affected udder milk samples are found to be resistant to various antimicrobial drugs (Table 1). These drugs include gentamicin, penicillin-G, streptomycin, ampicillin, oxytetracycline, and ciprofloxacin (Kumar et al. 2011; Singh et al. 2018).

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

S. aureus is a coccoid bacterium of Gram-positive nature. Being a coccoid organism means its cells are arranged in a grape-like irregular pattern. Usually, they reside in humans and animals as normal flora. It is found widely among humans with 30–40% of adults being asymptomatic carriers. It is considered a major culprit for infections in humans. It can produce a wide variety of infections in humans ranging from skin infections of and food poisoning of mild nature up to severe infections that can be fatal in the end. The discovery of methicillin-resistant *S. aureus* was primarily reported in 1961 shortly after the launching of antibacterial agents or antibiotics for clinical use (Conly and Johnston 2002; Taiwo 2011; Onemu and Ophori 2013; Kobayashi et al. 2015; Kong et al. 2016).

After that these pathogens began to multiply rapidly starting a global epidemic of Methicillin-resistant *S. aureus* (MRSA). This wave was felt in both community centers and healthcare systems (Weinstein and Fridkin 2001; Appelbaum 2006; Loomba et al. 2010). Isolates of MRSA from Denmark and the UK during the early phase of the 1960s were the very first indicators of an upcoming epidemic. The methicillin-resistant clone of *S. aureus* was introduced at that and since then it has emerged as a pathogen of utmost importance in human medicine that gives the premonition the era of antimicrobial resistance (Pinho et al. 2001; Lee 2003; Harkins et al. 2017). Despite methicillin being out of practice, and being replaced by isoxazolyl penicillins, particularly flucloxacillin in the UK, it is only out of the prescriptions for patients. Still, it has been a nightmare for the researchers. The acronym MRSA has still stayed as a nightmare reminding people of the upcoming dark times (Johnson 2011).

MRSA terminology is being used to characterize antibiotic resistance of bacteria to penicillins, cephalosporins, and carbapenems. Later, these bacteria have the tendency to develop resistance to quinolones, aminoglycosides, and macrolides (Okwu et al. 2012; Arunkumar et al. 2017; Adhikari et al. 2017). The origin of MRSA was a consequence of Staphylococcal Cassette Chromosome mec (SCCmec) genes being acquired by methicillin-susceptible *S. aureus* (MSSA). These genes, SCCmec, harbored the mecA gene. It was the gene that the penicillin-binding protein (PBP2a) encoded in itself. It is the main culprit that conferred resistance to all bacteria against β-lactam antibiotics (Rodríguez-Noriega et al. 2010; Arunkumar et al. 2017; Otto 2017; Sit et al. 2017).

SCCmec genes also contain the gene complex of cassette chromosome recombinases (ccr). The ccr genes mediate the integration and excision of SCCmec from the methicillin-resistant bacterium's chromosomes and into the chromosomes of susceptible bacteria, as it is composed of ccrC or a pair of ccrA and ccrB, and encode recombinases. The genes of ccr and surrounding genes make the complex of ccr genes. SCCmec contains a few genes and various other mobile genetic elements. These elements include insertion sequences, transposons, and plasmids along with ccr and mec gene complexes (Milheiriço et al. 2007; Okwu et al. 2016). Several different types of SCCmec numbered around eleven (I-XI) and some allotypes of the ccr gene complexes that are up to five (ccrAB1, ccrAB2, ccrAB3, ccrAB4, and ccrC) have been reported. Usually, hospital-acquired MRSA (HA-MRSA) is caused by SCCmec types I, II, III, VI, and VIII, while community-acquired MRSA (CA-MRSA) includes types IV, V, and VII while livestock-associated MRSA (LA-MRSA) includes types IX, X, and XI (Amirkhiz et al. 2015; Okwu et al. 2016).

Methicillin-resistance gene expression in *S. aureus* is commonly controlled and regulated by the mecI or blaI gene. These mecI and blaI gene repressors are under the control of the mecRI and blaRI transducers (Loomba et al. 2010). MRSA remains one of the major public health issues worldwide. It is also a therapeutic challenge as the treatment is costly due to the scarce availability of effective antibiotic drugs and their ever-increasing prices. The ever-varying epidemiological patterns of MRSA infections, changing resistance to antibiotics of common use, and involvement of community centers and hospitals in spreading infections have influenced the utilization and clinical results of presently available antibiotic drugs (Rodvold and McConeghy 2014).

Table 1: Antibiotic resistance (%) of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus agalactiae* (Singh et al. 2018).

No.	Antibiotics	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Streptococcus agalactiae*
1.	Chloramphenicol	31.03%	0%	0%	28%
2.	Co-trimoxazole	13.79%	60%	28%	13.9%
3.	Tetracycline	34.48%	37.03%	36%	67.44%
4.	Gentamicin	41.37%	0%	0%	4.6%
5.	Ofloxacin	0%	63%	40%	0%
6.	Erythromycin	31.03%	7.5%	76%	41.86%
7.	Amoxicillin	93.10%	100%	100%	93.03%
8.	Cephalexin	20.68%	7.5%	4%	23.25%
9.	Sparfloxacin	0%	33.33%	60%	9.3%
10.	Ciprofloxacin	0%	29.62%	24%	4.6%
11.	Vancomycin	31.03%	66%	36%	34.88%
12.	Teicoplanin	0%	66%	100%	30%
13.	Doxycycline	0%	26%	36%	90.69%
14.	Azithromycin	0%	0%	0%	0%
15.	Gatifloxacin	31.03%	29.62%	56%	29.03%

Vancomycin-Resistant *Staphylococcus aureus* (VRSA)

Vancomycin is an antibacterial agent and it works by inhibiting the production of bacterial cell walls. As a cell wall inhibitor, vancomycin performed its function by binding with precursors of a bacterial cell wall named Dalanyl-D-alanine C terminus. As a result of this binding, vancomycin stops the crosslinking through a process of transpeptidation. In more detail, in the process of inhibition of the production of the bacterial cell wall, vancomycin performs its function extracellularly and stops the biosynthesis of late-stage peptidoglycan. This extracellular activity of vancomycin results in the accumulation of precursors named UDP-linked MurNAc pentapeptide. The structure of the vancomycin complex basically consists of a number of hydrogen bonds present between the vancomycin peptide component and the bacterial cell wall residue D-Ala-D-Ala. So, if there is any condition of any other process which will disturb the binding of vancomycin drug with the D-Ala-D-Ala residue, it will affect the end results, for instance, the potency of the drug will decrease (Conly and Johnston 2002; Howden et al. 2010). The use of vancomycin against MRSA is increasing day by day and it is used in a huge amount for the treatment of MRSA, the outcomes of this are the emergence of vancomycin-intermediate and vancomycin-resistance *S. aureus* (VISA and VRSA) (Dhanalashmi et al. 2010). This situation is very alarming as *S. aureus* is a potential cause of infections in both hospitalized and non-hospitalized patients of the medical community (Weinstein and Fridkin 2001).

Many classes of *S. aureus* have developed resistance against vancomycin in different parts of the world. The main three classes which developed resistance include vancomycin-intermediate *S. aureus* (VISA), heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and the last one is vancomycin-resistant *S. aureus* (VRSA) (Appelbaum 2007). The first hospital stain of vancomycin-resistant *S. aureus* (VRSA) was reported in 2002 in a hospital in the United States (CDC 2002). When the trials are started to collect the vanA gene from vancomycin-resistant enterococci, the outcomes are in the form of the bursting of the vancomycin-resistant strain of *S. aureus* (VRSA) and the MIC value of vancomycin with this was higher than 16ug/mg (Chang et al. 2003; Howden et al. 2010; Gardete and Tomasz 2014).

Novel Strategies to Mitigate Resistant *S. aureus*

Different strategies can be wielded as a weapon to strike off the hydra of antibiotic resistance in the causative microbes of mastitis which include novel antibiotic development, new drug development, nanoparticles, and some herbal extracts too. In the past two decades, many research studies have been done on the herbal way to tackle methicillin-resistant *S. aureus* (MRSA). These herbal products are derived from various parts i.e., wood, leaves, stems, roots, etc, of specific plants with the help of specific extraction agents like ethanol or methanol. These products have shown specific antimicrobial properties which enable them to tackle Methicillin-resistant *S. aureus* (Table 2) (Shopsin et al. 1999; Yang-Hwei et al. 2008; Hamal et al. 2009; He et al. 2014; Jesline et al. 2015; Natarajan et al. 2015; Zaatout 2022).

Different nanoparticles withhold antimicrobial capacities of various intensities depending upon the rival microbe, population of the microbes, resistance properties of the microbe and the environment of the microbial population where the interaction occurs between the two (Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013; Hozyen et al. 2019). These nanoparticles can be used against various resistant causative microorganisms of both clinical and subclinical mastitis (Table 3).

Conclusion

Antimicrobial resistance is a burning issue around the globe these days. As there is a lack of novel drugs and the development of new antibiotics isn't happening that often, antimicrobial resistance knocks down the only shield held

Table 2: Botanical products that have antimicrobial properties to mitigate MRSA.

No.	Plant name	Family	Part of the plant used	References
1.	*Acacia catechu* (L.f.) Willd	Fabaceae	Wood	(Voravuthikunchai and Kitpipit 2005; CABI 2018a)
2.	*Impatiens balsamina*	Balsaminaceae	Leaves	(Voravuthikunchai and Kitpipit 2005; CABI 2018b)
3.	*Walsura robusta*	Meliaceae	Wood	(Voravuthikunchai and Kitpipit 2005; Arefin et al. 2011)
4.	*Butea monosperma Lam.*	Fabaceae	Leaves	(Sahu and Padhy 2013)
5.	*Acacia albida* Del.	Fabaceae	Stem Bark	(Aliyu et al. 2008)
6.	*Anchomanes difformis* Engl.	Araceae	Roots	(Aliyu et al. 2008)
7.	*Boscia senegalensis* Del.	Capparidaceae	Roots	(Aliyu et al. 2008)
8.	*Moringa oleifera* Lam.	Moringacceae	Leaves	(Aliyu et al. 2008)
9.	*Mormodica basalmina* Linn.	Cucurbitaceae	Whole Plant	(Aliyu et al. 2008)
10.	*Nymphaea lotus* Linn.	Nymphaeaceae	Leaf	(Akinjogunla et al. 2010)
11.	*Pavetta crassipes* K. Schum.	Rubiaceae	Roots	(Aliyu et al. 2008)
12.	*Phyllanthus amarus* Schum. Thonn.	Euphorbiaceae	Roots	(Aliyu et al. 2008)
13.	*Vernonia blumeoides* Hook. f.	Asteraceae	Aerial part	(Aliyu et al. 2008)
14.	*Machilus salicina* Hance.	Lauraceae	TBL	(Zuo et al. 2012)
15.	*Selaginella tamariscina* (Seauv.) Spring.	Selaginellaceae	Whole plant	(Zuo et al. 2012)
16.	*Celastrus orbiculatus* Thunb.	Celastraceae	Vane	(Zuo et al. 2012)
17.	*Carex prainii* C.B. Clarke	Cyperaceae	Whole plant	(Zuo et al. 2012)
18.	*Embelia burmf.*	Myrsinaceae	Leaves	(Zuo et al. 2012)
19.	*Withania somnifera L.*	Solanaceae	Roots & Leaves	(Heyman et al. 2009; Uddin et al. 2012; Nefzi et al. 2016)

Table 3: Nanoparticles that have antimicrobial properties to mitigate the causative & resistant bacteria of subclinical and clinical mastitis.

No.	Microbe	Nanoparticle	Reference
1.	*S. aureus*	Zinc Nanoparticles	(Namasivayam et al. 2015)
		Titanium Nanoparticles	(Shopsin et al. 1999; Yang-Hwei et al. 2008; Hamal et al. 2009)
		Iron Oxide	(Behera et al. 2012; Ismail et al. 2015; Masadeh et al. 2015)
		Cobalt Iron Oxide	(Kooti et al. 2015)
		Copper Nanoparticles	(Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013)
		Magnesium Oxide	(Jeong et al. 2007)
		Calcium Oxide	(Jeong et al. 2007)
2.	*E. coli*	Zinc Nanoparticles	(Namasivayam et al. 2015)
		Titanium Nanoparticles	(He et al. 2014; Jesline et al. 2015; Natarajan et al. 2015)
		Iron Oxide	(Behera et al. 2012; Ismail et al. 2015; Masadeh et al. 2015)
		Silicon & Silver Oxide	(Kooti et al. 2015)
		Copper Nanoparticles	(Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013)
		Magnesium Oxide	(Jeong et al. 2007)
		Calcium Oxide	(Jeong et al. 2007)
3.	*Klebsiella pneumoniae*	Copper Nanoparticles	(Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013)
		Iron Oxide	(Behera et al. 2012; Ismail et al. 2015; Masadeh et al. 2015)

against any upcoming disease outbreak or pandemic. There has been a massive rise in the antimicrobial resistance in the causative agents of subclinical and clinical mastitis, among which Methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *S. aureus* (VRSA) are the most common or prevalent ones. Different strategies can be wielded as a weapon to strike off the hydra of antibiotic resistance in the causative microbes of mastitis which include novel antibiotic development, new drug development, nanoparticles, and some herbal extracts too. In the past two decades, many research studies have been done on the herbal way to tackle methicillin-resistant *S. aureus* (MRSA). These herbal products are derived from various parts i.e., wood, leaves, stems, roots, etc., of specific plants with the help of specific extraction agents like ethanol or methanol. These products have shown specific antimicrobial properties which enable them to tackle Methicillin-resistant *S. aureus.* Different nanoparticles withhold antimicrobial capacities of various intensities depending upon the rival microbe, the population of the microbes, the resistance properties of the microbe, and the environment of the microbial population where the interaction occurs between the two. These nanoparticles can be used against various resistant causative microorganisms of both clinical and subclinical mastitis.

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