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Screening Of Multidrug-Resistant Bacteria In Bovine Infectious Mastitis Isolates

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Abstract:
Background*: Antibiotic resistance has negative implications for treating infections in humans and animals. Rapid identification of the pathogen causing the disease is one of the determining factors for effective treatment. This study aimed to use MALDI-TOF MS to investigate multidrug-resistant bacterial strains in bacterial isolates from infectious bovine mastitis.*
Materials and Methods*: Fifty-four milk samples from cows with subclinical mastitis were sown on sheep blood agar, and the bacterial isolates were identified by MALDI-TOF MS. The disk diffusion test verified susceptibility to antimicrobials.*
Results*: The largest number of isolates were resistant to OXA (69%), followed by CFO (52%), AMP (48%), PEN (44%) and AMO (41%). The lowest number of resistant strains was observed against the antibiotic VAN. Although less significant, some bacterial strains were resistant to carbapenems. All the multidrug-resistant strains were identified on the MALDI-TOF MS system, including Staphylococcus aureus and non-aureus, Enterobacter asburiae, Enterobacter cloacae and Enterococcus faecalis. The species Staphylococcus aureus was detected most frequently.*
Conclusion: *The detection of bacteria resistant to two or more antibiotics usually used to treat bovine mastitis indicates the need to look for alternative treatments to control this disease.*
Key Word*: Vancomycin; Cabapenems; Staphylococcus aureus; non-aureus Staphylococcus.*

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I. Introduction

Infectious bovine mastitis is considered an important disease affecting dairy herds all over the world. It is a multifactorial disease defined as inflammation of the mammary gland (Viguier et al., 2009). Conventionally, antimicrobial therapy is used to treat infectious bovine mastitis. The scientific literature points to the use of different types of antimicrobials in the treatment of intramammary infections caused by bacteria (Langoni et al., 2017; Ribeiro et al., 2016; Ribeiro, 2008). However, the success of treating infectious bovine mastitis caused by bacteria is variable. The resistance of bacteria to the antimicrobials routinely used is one of the determinants of successful treatment (Dereti, Zanela, Méndez, 2022).

Antibiotic resistance is considered one of the main global threats today, with negative implications for treating infections in humans and animals (Fernandes Prates et al., 2020). Rapid identification of the pathogen causing the infection is one of the determining factors for effective treatment. The use of MALDI-TOF MS to identify microorganisms has already become a reality in the routine of many microbiology laboratories, due to all the advantages it offers, and is only not more widely used due to the high cost of the equipment (De Carolis et al, 2014; Croxatto, Prod'hom, Greub, 2012).

Several studies point to MALDI-TOF MS as a successful tool for detecting mastitis pathogens in bovine milk samples (Werner et al., 2012; Esener et al., 2018; Jahan et al., 2021) and even in cases of antimicrobial resistance associated with bovine mastitis (Esener et al., 2021). These studies highlight the potential applications

of the MALDI-TOF MS system in the diagnosis of infectious bovine mastitis, contributing to the epidemiological investigation of the disease and the monitoring of multidrug-resistant bacteria causing bovine mastitis.

Given the above, this study aimed to use MALDI-TOF MS to investigate multidrug-resistant bacterial strains in bacterial isolates from infectious bovine mastitis on dairy farms in northern Minas Gerais, Brazil.

II. Material And Methods

Collection of milk samples and bacterial isolates

Fifty-four milk samples from cows with subclinical mastitis were collected from teats on farms in northern Minas Gerais, Brazil. All procedures carried out in vivo were previously approved by the Ethics Committee on the Use of Animals under protocol number CEUA 90/2018. Cases of clinical and subclinical mastitis were diagnosed using the cup test and the California Mastitis Test (CMT), respectively (Costa et al., 2018; Schalm; Noorlander, 1957).

Milk samples were collected from each teat in sterile vials, packed in cool boxes with ice and sent to the Animal Health Laboratory located at the Agricultural Sciences Research Center of the Federal University of Minas Gerais - Brazil (CPCA - ICA/UFMG). After the samples were sown on sheep blood agar (5%V/V) using the depletion technique, the isolated colonies were characterized in terms of color, size and the formation of hemolysis halos and subsequent Gram staining.

Identification of infectious bovine mastitis bacteria by MALDI-TOF MS

The isolated colonies were identified by mass spectrometry (MALDI-TOF MS), according to Souza et al. (2019). Briefly, each of the isolated colonies was added to a steel plate with 1 µl of formic acid (70%) and 1 µl of MALDI-TOF MS matrix, which is a saturated solution of a-cyano-4-hydroxycinnamic acid (Bruker Daltonics, Bremen, Germany). The plate was left to dry in the open air.

To obtain the spectra of interest, a MicroFlex LT mass spectrometer (Bruker Daltonics) with a 60 Hz nitrogen laser was used. Up to 240 laser shots are fired in spiral movements to collect 40 shot steps for each voltage point. Following the manufacturer's recommendations, the parameters for detecting the mass range were set as shown in the table below:

Table 1: Parameters for detecting the mass range, according to the manufacturer's recommendations

Parameters	Definition
Mass range	1960 to 20,137 m/z
Ion source 1 v	19.99 kv
Ion source voltage 2 kv	18.24 kv
Lens voltage	6.0 kv

The scores assigned for the identification of species and genera in real-time were ≥2 indicating identification at the species level, ≥1.7 and <2 indicating identification at the genus level and <1.7 indicating no reliable identification.

Determination of antimicrobial susceptibility

The bacterial isolates were subjected to the antimicrobial susceptibility test by disk diffusion (CLSI, 2018). The following antimicrobials were defined for these tests: ampicillin 10µg (AMP), penicillin 10U (PEN), sulfazotrin 25µg (SUT), ciprofloxacin 5µg (CIP), kanamycin 30µg (KAN), cephalexin 30µg (CFE), gentamicin 10µg (GEN), ceftazidineµg (CAZ), amoxicillin + clavulanate 30µg (AMC), cefoxitin 30µg (CFO), meropenem 10µg (MER), nitrofurantoin 300µg (NIT), cephalothinµg (CFL), vancomycin 30µg (VAN), amoxicillin 10µg (AMO), oxacillin 1µg (OXA) (CLSI, 2018). The classification of methicillin-resistant isolates was defined by resistance to oxacillin and/or cefoxitin, as recommended by CLSI (2018).

Data analysis

The results were subjected to descriptive statistics through the distribution of relative and absolute frequencies for the microbiological findings. The frequency of resistance to antimicrobials alone and in combination was assessed. MALDI-TOF MS scores were used to create heat map graphs showing the antibiotic resistance profile of each microorganism.

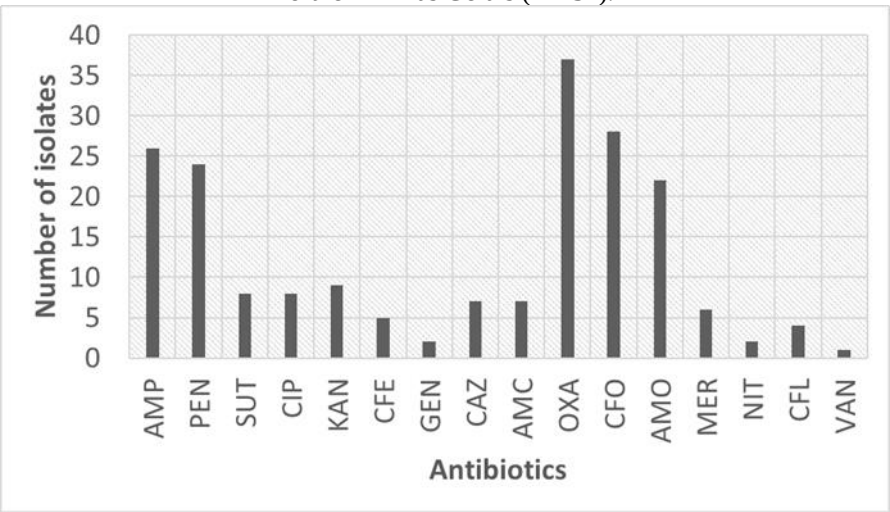
III. Result And Discussion

As can be seen in Figure 1, the largest number of isolates were resistant to OXA (69%), followed by CFO (52%), AMP (48%), PEN (44%) and AMO (41%). The lowest number of resistant strains was observed against the antibiotic VAN. In a study that investigated the antimicrobial sensitivity of bacterial strains isolated in milk samples from goats with subclinical mastitis, there was a higher rate of antimicrobial resistance to the antibiotics penicillin (73%), ampicillin (52.4%) and oxacillin (52.4%) (de Castro et al., 2017). In the state of São

Paulo, greater resistance of bacterial isolates to penicillin, oxacillin and ampicillin was also observed, with frequencies ranging from 55.5% to 81.8% (Salaberry et al., 2016). Acosta et al. (2016) observed that penicillin, ampicillin, amoxicillin and neomycin are the antimicrobials to which mastitis-causing microorganisms are most resistant.

The data presented supports the warning signs of the resistance of microorganisms causing infectious bovine mastitis to different antibiotics. Oxacillin-resistant bacterial strains commonly observed in cases of mastitis represent a considerable public health problem, as in addition to being multidrug-resistant, they can express important virulence factors (Fitzgerald, 2014; Monaco et al., 2017). Resistance to this antibiotic is related to various factors such as the presence of the mecA gene, which makes microorganisms intrinsically resistant to other antimicrobials as well (Carvalho, Berezin, 2004).

Figure 1: Antimicrobial resistance of multidrug-resistant bacteria isolates associated with bovinemastitis in northern Minas Gerais (n = 54).



Cefoxitin is a potent inducer of the mecA gene regulatory system, as it has a high affinity for PBP2 (penicillin-binding protein) (Cauwelier et al., 2004), which is an essential protein for cell wall synthesis and bacterial growth in the presence of the antimicrobial (Mangueira, 2012). Resistance to cefoxitin and/or oxacillin can also be considered methicillin resistance (CLSI, 2018), and is used in tests to detect methicillin-resistant *Staphylococcus aureus* (MRSA). Therefore, this highlights a worrying situation, since methicillin would be a drug of choice against penicillinase-producing strains.

After oxacillin and cefoxitin, antibiotics from the penicillin class have induced resistance in a greater number of bacterial isolates. Ampicillin, penicillin, amoxicillin and tetracycline are the antibiotics most commonly used to treat mastitis and other infections in cattle, but they can have limited efficacy (Dos Santos et al., 2011) due to the various resistance mechanisms involved. The classic mechanism of resistance to ampicillin, penicillin and amoxicillin is due to the production of β-lactamases, which inactivate the antibiotics by hydrolyzing the β-lactam ring of penicillins (Dzidic et al., 2008).

Although the number of bacterial strains resistant to carbapenems and vancomycin was low, it is a worrying situation, since these are not drugs that are commonly used in animal production. They are considered reserve drugs and, even in humans, should be administered as a last resort when there is no other resource to be used (WHO, 2017). The resistance profile observed in this study suggests cross-contamination between humans and animals and/or the transfer of resistance genes between microorganisms (Fu et al., 2019). The importance of these findings from a public health point of view is emphasized, since their use in veterinary medicine is restricted, especially in production animals, to avoid the emergence of resistance, which in the present study was observed for strains of *Staphylococcus aureus* and non-aureus.

As a result of the above and the need to develop more effective control measures for infectious bovine mastitis, it is important to carry out studies to identify resistant microorganisms so that strategies can be developed to eliminate them. In this context, MALDI-TOF MS is presented as a favorable technique for identifying microorganisms.

Identification of the microbial strains using MALDI-TOF MS was possible down to the species level. All multidrug-resistant strains were identified on the MALDI-TOF MS system, including *Staphylococcus aureus* and non-aureus, *Enterobacter asburiae*, *Enterobacter cloacae* and *Enterococcus faecalis*. The *Staphylococcus aureus* species was detected most frequently (Figure 2).

MALDI-TOF MS mass spectrometry has been successfully used to identify a wide variety of bacterial species (Clark et al., 2013). Studies using the MALDI-TOF MS technique have found correct identification of 99.8% for genus and 98.2% for species (Faron et al., 2015). For *staphylococcal* species, 99.3% of the species were correctly identified, as well as all the subspecies studied (Spanu et al., 2011). Other studies suggest the use of MALDI-TOF MS for the identification and determination of antimicrobial-resistant microorganisms that cause bovine mastitis (Esener et al., 2021), highlighting the accuracy of the technique. This highlights the promising nature of the technique for the diagnosis of bovine mastitis and potentially for the surveillance of antimicrobial-resistant microorganisms in bovine mastitis (Zhang et al., 2024).

Figure 2: Multidrug-resistant bacterial species in bovine infectious mastitis isolates in northern Minas Gerais identified by MALDI-TOF MS (n = 54).

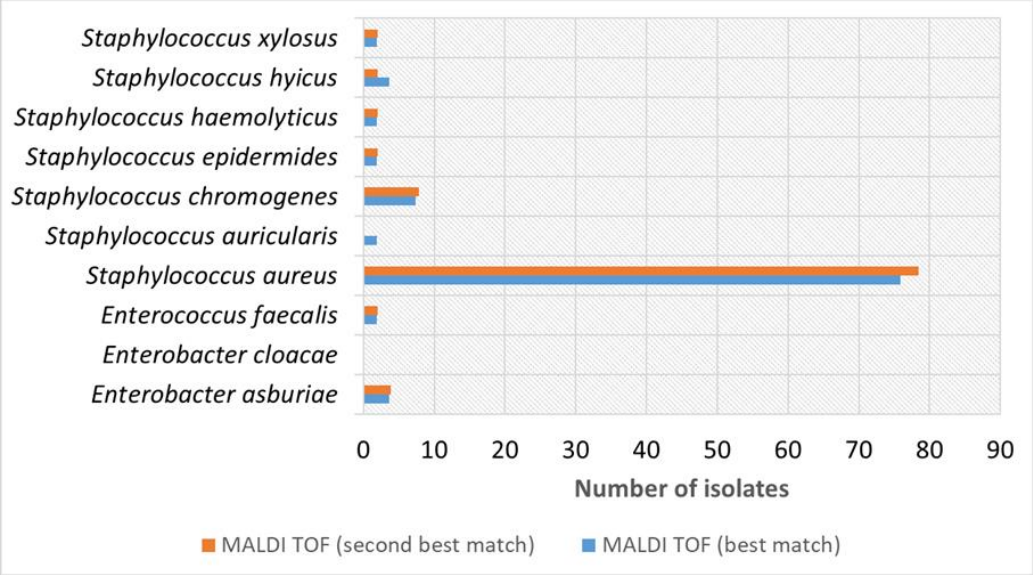


Table 2 shows the bacterial species identified using the MALDI-TOF MS system. The *Enterobacter asburiae* species was detected by MALDI-TOF MS with scores above 2.3. The *Enterobacterales* order is present in various situations and can be found in the human and animal gastrointestinal microbiota (Mezzatesta; Gona; Stefani, 2012), or as a cause of severe infections with a high capacity for resistance to antimicrobials, being of great importance to public health. Within the hospital environment, in human medicine, they are commonly isolated in tracheal secretion, blood culture, uroculture and sputum (Basso et al., 2016; Magalhães et al., 2014).

Enterococcus faecalis (MALDI-TOF MS score > 2.2) stands out among the microorganisms that cause environmental bovine mastitis. This bacterium is usually found in the tonsils, intestines, skin and manure of the animal, and is known to form biofilm, a key virulence factor in most infections (Elhadidy, Zahran, 2014). *Enterococci* are one of the most frequently isolated bacteria from mild and acute clinical cases of bovine mastitis (Werner et al., 2012). Even with the considerable progress of mastitis control programs, these procedures remain ineffective against environmental pathogens such as *Enterococcus faecalis* which represents an important microorganism causing environmental mastitis (Yang et al., 2019).

Table 2 - Species of multidrug-resistant bacteria identified using the MALDI-TOF MS system

Microorganisms	MALDI-TOF MS			
	Best match		Second best match	
	Minimum score	Maximum score	Minimum score	Maximum score
Enterobacter asburiae	2.342	2.374	2.307	2.34
Enterococcus faecalis	2.271	2.271	2.27	2.27
Staphylococcus aureus	1.75	2.632	1.786	2.624
Staphylococcus auricularis	1.857	1.857	-	-
Staphylococcus chromogenes	2.029	2.343	1.838	2.322
Staphylococcus epidermides	2.016	2.016	2.004	2.004
Staphylococcus haemolyticus	2.223	2.223	2.139	2.139
Staphylococcus hyicus	1.932	2.006	1.905	1.905
Staphylococcus xylosus	2.043	2.043	2.001	2.001

In addition to bacteria from the Enterobacter and Enterococcus genera, the *Staphylococcus* genus prevailed among the microorganisms detected. *Staphylococcus aureus*, with a MALDI-TOF MS detection score of over 2.6, is a coagulase-positive microorganism and is present in different types of vectors, such as animal skin, teats, tonsils, cattle bedding and milker's hands, which favor its spread and high contamination (Gao et al., 2012). The increase in the presence of Staphylococcus aureus isolated from bovine mastitis with multidrug-resistant characteristics is a major public health problem, as it increases treatment costs and the morbidity of the disease (Bonsaglia et al., 2018).

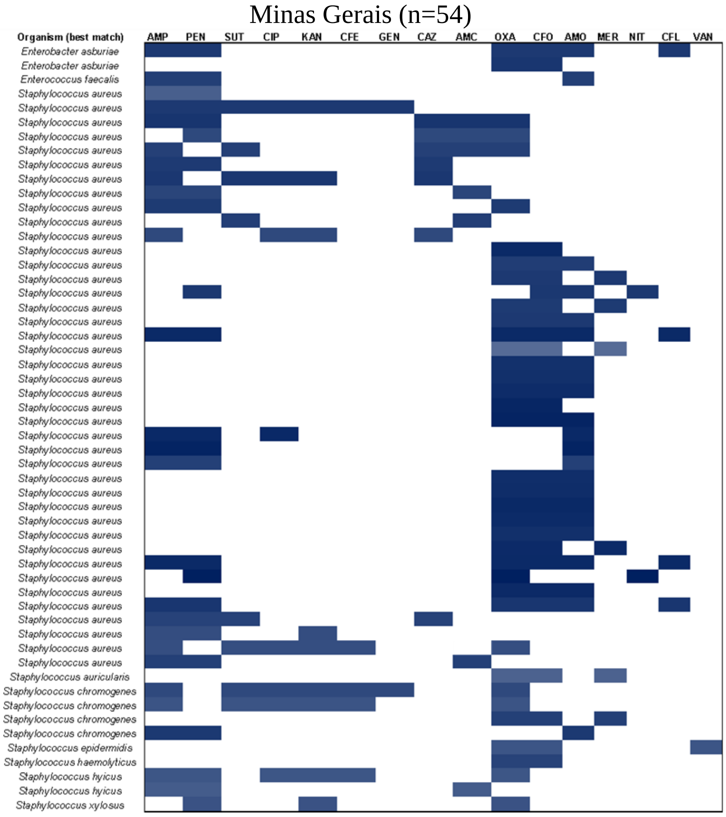
Non-aureus, coagulase-negative *Staphylococcus* agents, with scores ranging from 1.8 to 2.3, are considered minor mastitis agents because they cause few changes to the udder and milked milk of infected animals, but can cause major losses in situations of high prevalence (Thorberg, 2008).

Figure 3 shows the resistance profiles of the bacterial species identified by MALDI-TOF MS. The two isolates of *Enterobacter asburiae* were resistant to OXA and CFO. However, only one of the isolates showed resistance to AMP, PEN, AMO and CFL, suggesting a mutation between strains of the same species. A study of the identification and sensitivity profile of *Enterobacter* spp. isolated from raw bovine milk showed a frequency of strains resistant to various antimicrobials and the presence of the blaTEM gene in the *Enterobacter* genus (Alves et al., 2015).

Previous studies have shown an increase in antimicrobial resistance among different microorganisms, especially the *Staphylococcus* and *Enterococcus* genera (Mendes et al., 2002). *Enterococcus faecalis* was found to be resistant to the antibiotics AMP, PEN and AMO (Figure 3). Literature reports show complications arising from enterococcus infections since increased resistance has been observed to different antimicrobials routinely prescribed for Gram-positive cocci, particularly penicillin. In addition, the ability of these microorganisms to acquire resistance genes via transposons or plasmids has been recorded (Medeiros et al., 2014).

Among the multidrug-resistant microorganisms identified by MALDI-TOF MS, *Staphylococcus aureus* is the most common agent related to contagious bovine mastitis, with the potential to develop resistance factors to almost all the antimicrobial agents analyzed in this study. Figure 3 shows that different isolates of *Staphylococcus aureus* have different multidrug resistance profiles, which suggests mutations in their genes and/or the acquisition of resistance genes from other bacteria of the same species, or possibly from other species (Alós, 2015; Rodríguez-noriega et al., 2013). Only the antimicrobial VAN was effective against all strains of *Staphylococcus aureus*. This bacterial species is susceptible to the action of various drugs against Gram-positive bacteria. However, it is also recognized for its high capacity to develop resistance to all of them (Alós, 2015; Linares-rodríguez; Martínez menéndez, 2005).

Figure 3: Screening of multidrug-resistant bacterial species in bovine infectious mastitis isolates in northern Minas Gerais (n=54)



Most of the antibiotics were not effective against any of the coagulase-negative, non-aureus *Staphylococcus*. Only the antimicrobials CAZ, NIT and CFL were effective against this group of *Staphylococcus* (Figure 3). Vancomycin was once considered the treatment of choice for infections caused by this type of microorganism. However, many cases of resistance by microorganisms from the *enterococci* and *staphylococci* groups have been recorded in the literature (Marshall et al., 1998; Szarapinska-Kwaszewska, Farkas, 2003). These occurrences have led to a reduction in the use of this drug (Szewczyk et al, 2000).

Figures 4 and 5 show the two highest-scoring strains of each bacterial species, identified according to the NCBI library. Figure 4 shows the non-aureus bacteria and Figure 5 the *Staphylococcus aureus* species.

Figure 4: Identification of multidrug-resistant bacterial strains (non-aureus *Staphylococcus*) in bovine infectious mastitis isolates in northern Minas Gerais (n=49), in agreement with NCBI data

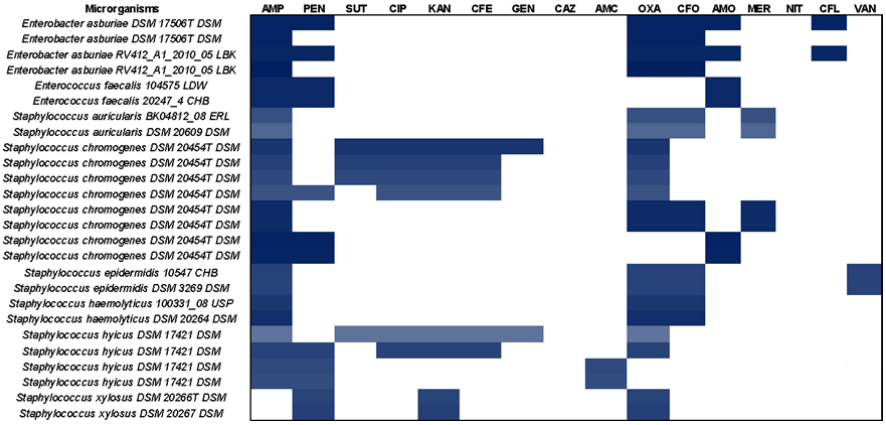
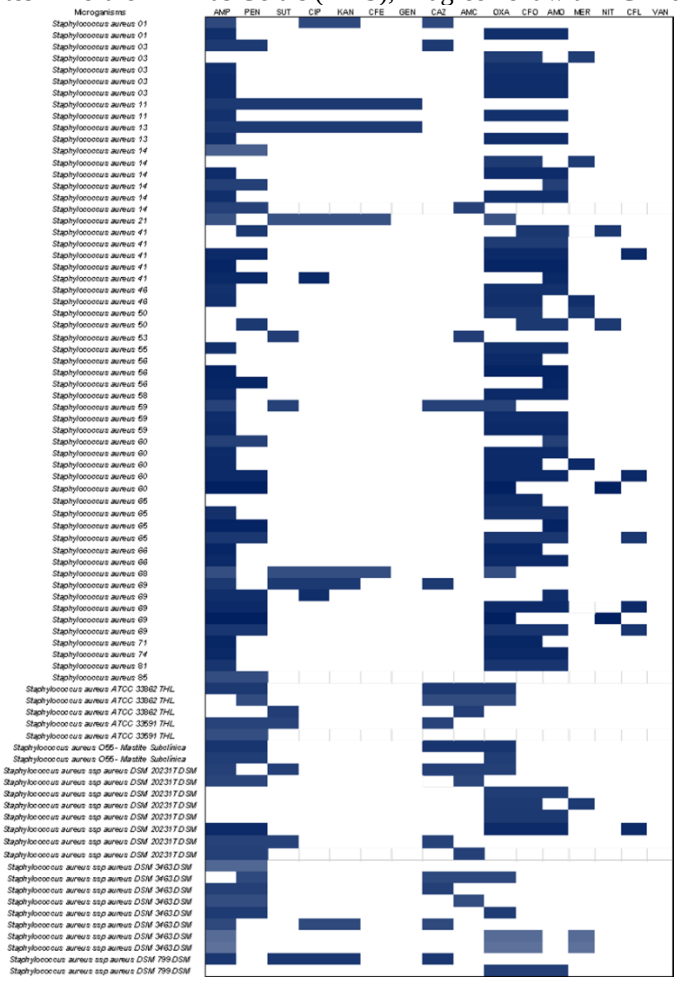


Figure 5: Identification of multidrug-resistant *Staphylococcus aureus* strains in bovine infectious mastitis isolates in northern Minas Gerais (n=49), in agreement with NCBI data



The results obtained contribute to a better understanding of the occurrence of multidrug-resistant microorganisms on dairy farms in northern Minas Gerais, Brazil. This makes it easier to target treatment and control strategies for bovine mastitis.

IV. Conclusion

The number of multi-resistant bacteria is constantly increasing. The detection of bacteria resistant to two or more antibiotics usually used to treat bovine mastitis indicates the need to look for alternative treatments to control this disease.

Although the majority of microorganisms resistant to antimicrobials are *Staphylococcus aureus*, non-aureus bacteria were also identified, which, although not very prevalent in bovine mastitis, showed a multidrug-resistant profile.

Many bacterial strains have become resistant to even the most modern antibiotics routinely used in human medicine, which is a worrying issue from a public health point of view.

Rapid identification of resistant bacterial species is of great importance for human and animal health, biotechnology applications and the pharmaceutical industry. From this perspective, the MALDI-TOF MS technique is promising for contributing to the diagnosis of bovine mastitis and can be used routinely to identify multidrug-resistant pathogens after isolation by culture of samples collected on dairy farms.

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References

[1]. Acosta Ac, Silva Lbgd, Medeiros Es, Et Al. Mastitis In Ruminants In Brazil. Pesquisa Veterinária Brasileira. 2016;36(7):565-573.
[2]. Alós Ji. Resistencia Bacteriana A Los Antibióticos: Una Crisis Global. Enfermedades Infecciosas Y Microbiología Clínica. 2015;33(10):692-699.
[3]. Alves Ts, Siqueira Ak, Ferraz Mmg, Et Al. Identification And Sensitivity Profile Of Enterobacter Spp. Isolated From Raw Bovine Milk. Veterinária E Zootecnia. 2015;22(1):114-122.
[4]. Basso Me, Pulcinelli Rsr, Aquino Arc, Et Al. Prevalence Of Bacterial Infections In Patients Admitted To An Intensive Care Unit (Icu). Rbac. 2016;48(4):383-388.
[5]. Bonsaglia Ecr, Silva Nc, Rossi Bf, Et Al. Molecular Epidemiology Of Methicillin-Susceptible Staphylococcus Aureus (Mssa) Isolated From Milk Of Cows With Subclinical Mastitis. Microbial Pathogenesis. 2018;124:130-135.
[6]. Carvalho Ce, Berezin En, Pistelli Ip, Et Al. Sequential Microbiological Monitoring Of Tracheal Secretions From Intubated Patients In A Pediatric Icu. Jornal De Pediatria. 2004;81(1):23-29.
[7]. Cauwelier B, Gordts, B, Descheemaecker P, Et Al. Evaluation Of A Disk Diffusion Method With Cefoxitin (30 Mg) For Detection Of Methicillin-Resistant Staphylococcus Aureus. European Journal Of Clinical Microbiology And Infectious Diseases. 2004;23:389-392.
[8]. Clark Ae, Kaleta Ej, Arora A, Et Al. Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry: A Fundamental Shift In The Routine Practice Of Clinical Microbiology. Clinical Microbiology Reviews. 2013;26(3):547-603.
[9]. Clsi- Clinical And Laboratory Standards Institute: Performance Standards For Antimicrobial Susceptibility Testing. 28th Ed, Clsi Supplement M100. Wayne, Pa: Clinical And Laboratory Standards Institute. 2018.
[10]. Costa Fn, Belo No, Costa Ea, Et Al. Frequency Of Enterotoxins, Toxic Shock Syndrome Toxin-1, And Biofilm Formation Genes In Staphylococcus Aureus Isolates From Cows With Mastitis In The Northeast Of Brazil. Tropical Animal Health And Production. 2018;50:1089-1097.
[11]. Croxatto A, Prodhom G, Greub G. Applications Of Maldi-Tof Mass Spectrometry In Clinical Diagnostic Microbiology. Fems Microbiology Reviews. 2012;36(2):380-407.
[12]. De Carolis E, Vella A, Vaccaro L, Et Al. Application Of Maldi-Tof Mass Spectrometry In Clinical Diagnostic Microbiology. The Journal Of Infection In Developing Countries. 2014;8(9):1081-1088.
[13]. De Castro Lk, Lima M.C, Pifano Nk, Et Al. Perfil Antimicrobiano De Staphylococcus Aureus Obtidos De Mastite Caprina. Revista Científica Univiçosa. 2018;9(1):481-482.
[14]. Dereti Rm, Zanela Mb, Méndez Mg. Uso Prudente De Antimicrobianos Para Tratamento Da Mastite Bovina, Embrapa, Pelotas, Rs, 2022.
[15]. Dos Santos Ll, Da Costa Gm, Pereira Up, Et Al. Clinical And Subclinical Mastitis In Dairy Cattle Caused By Coagulase-Negative Staphylococcus. Revista Do Instituto Adolfo Lutz. 2011;70(1):1-7.
[16]. Džidić S, Šušković J, Kos B. Antibiotic Resistance Mechanisms In Bacteria: Biochemical And Genetic Aspects. Food Technology & Biotechnology. 2008;46(1):11-21.
[17]. Elhadidy M, Zahran E. Biofilm Mediates Enterococcus Faecalis Adhesion, Invasion And Survival Into Bovine Mammary Epithelial Cells. Letters In Applied Microbiology. 2014;58(3):248-254.
[18]. Esener N, Green Mj, Emes Rd, Et Al. Discrimination Of Contagious And Environmental Strains Of Streptococcus Uberis In Dairy Herds By Means Of Mass Spectrometry And Machine-Learning. Scientific Reports. 2018;8(17517):1-12.
[19]. Esener N, Maciel-Guerra A, Giebel K, Et Al. Mass Spectrometry And Machine Learning For The Accurate Diagnosis Of Benzylpenicillin And Multidrug Resistance Of Staphylococcus Aureus In Bovine Mastitis. Plos Computational Biology. 2021;17(6):1-28.

[20]. Faron Ml, Buchan Bw, Hyke J, Et Al. Multicenter Evaluation Of The Bruker Maldi Biotyper Ca System For The Identification Of Clinical Aerobic Gram-Negative Bacterial Isolates. Plos One. 2015;10(11):1-13.
[21]. Prates Fi, Silva Gf, Fernandes Ra, Et Al. Hazards Provocated By Bacterial Resistance: A Worldwide Public Health Problem. Brazilian Journal Of Surgery & Clinical Research. 2020;32(2):121-138.
[22]. Fitzgerald Jr. Evolution Of Staphylococcus Aureus During Human Colonization And Infection. Infection, Genetics And Evolution. 2014;21:542-547.
[23]. Fu P, Tang Y, Li G, Et Al. Pandemic Spread Of Blakpc-2 Among Klebsiella Pneumoniae St11 In China Is Associated With Horizontal Transfer Mediated By Incfii-Like Plasmids. International Journal Of Antimicrobial Agents. 2019;54(2):117-124.
[24]. Gao J, Ferreri M, Yu F, Et Al. Molecular Types And Antibiotic Resistance Of Staphylococcus Aureus Isolates From Bovine Mastitis In A Single Herd In China. The Veterinary Journal. 2012;192(3):550-552.
[25]. Jahan Na, Godden Am, Royster R, Et Al. Evaluation Of The Matrix-Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry (Maldi-Tof Ms) System In The Detection Of Mastitis Pathogens From Bovine Milk Samples. Journal Of Microbiological Methods. 2021;182(106168).
[26]. Langoni H, Salina A, Oliveira Gc, Et Al. Considerações Sobre O Tratamento Das Mastites. Pesquisa Veterinária Brasileira. 2017;37(11):1261-1269.
[27]. Linares-Rodríguez Jf, Martínez-Menéndez Jl. Martínez-Menéndez, José Luis. Antimicrobial Resistance And Bacterial Virulence. Enfermedades Infecciosas Y Microbiologia Clinica. 2005;23(2):86-93.
[28]. Machado Ls, De Abreu Es, Pussente Cg, Et Al. Incidência E Perfil De Sensibilidade E Resistência Das Estirpes Bacterianas Isoladas Das Hemoculturas De Um Hospital Oncológico. Revista Científica Da Faminas. 2014;10(2):40-53.
[29]. Mangueira Evc. Avaliação Da Resistência A Antibióticos Beta-Lactâmicos Em Isolados De Staphylococcus Spp. Tese De Doutorado. 2012.
[30]. Marshall Sa, Wilke Ww, Pfaller Ma, Et Al. Staphylococcus Aureus And Coagulase-Negative Staphylococci From Blood Stream Infections: Frequency Of Occurrence, Antimicrobial Susceptibility, And Molecular (Meca) Characterization Of Oxacillin Resistance In The Scope Program. Diagnostic Microbiology And Infectious Disease. 1998;30(3):205-214.
[31]. Medeiros Aw, Pereira Ri, Oliveira Dvd, Et Al. Molecular Detection Of Virulence Factors Among Food And Clinical Enterococcus Faecalis Strains In South Brazil. Brazilian Journal Of Microbiology. 2014;45(1):327-332.
[32]. Mendes C, Sinto S. I, Hsiung A, Et Al. Atividade Antimicrobiana In Vitro De Quinupristina/Dalfopristina Para Cocos Gram-Positivos Isolados De Cinco Centros Brasileiros: Resultado Do Estudo De Vigilância L-Smart. Jornal Brasileiro De Patologia E Medicina Laboratorial. 2002;38(3):191-197.
[33]. Mezzatesta Ml, Gona F, Stefani S. Enterobacter Cloacae Complex: Clinical Impact And Emerging Antibiotic Resistance. Future Microbiology. 2012;7(7):887-902.
[34]. Monaco, M., Araujo Fp, Cruciani M, Et Al. Worldwide Epidemiology And Antibiotic Resistance Of Staphylococcus Aureus. Current Topics In Microbiology And Immunology. 2017;21-56.
[35]. Ribeiro Mg, Langoni H, Domingues Pf, Et Al. Mastite Em Animais Domésticos. Doenças Infecciosas Em Animais De Produção E De Companhia. Roca, Riode Janeiro. 2016;1155-1205.
[36]. Ribeiro Mg. Princípios Terapêuticos Na Mastite Em Animais De Produção E De Companhia. Manual De Terapêutica Veterinária. 3rd Ed. Roca, São Paulo. 2008;759-771.
[37]. Rodríguez-Noriega E, León-Garnica G, Petersen-Morfín S, Et Al. The Evolution Of Bacterial Resistance In Mexico, 1973-2013. Biomédica. 2014;34:181-190.
[38]. Salaberry Srs, Saidenberg Abs, Zuniga E, Et Al. Microbiological Analysis And Sensitivity Profile Of Staphylococcus Spp. In Subclinical Mastitis Of Dairy Goats. Arquivo Brasileiro De Medicina Veterinária E Zootecnia. 2016;68(2):336-344.
[39]. Schalm Ow, Noorlander Do. Experiments And Observations Leading To Development Of The California Mastitis Test. Journal Of The American Veterinary Medical Association. 1957;130(5):199-204.
[40]. Souza Gáad, De Almeida Ac, Xavier Mas, Et Al. Characterization And Molecular Epidemiology Of Staphylococcus Aureus Strains Resistant To Beta-Lactams Isolated From The Milk Of Cows Diagnosed With Subclinical Mastitis. Veterinary World. 2019;12(12):1931-1939.
[41]. Spanu T, De Carolis E, Fiori B, Et Al. Evaluation Of Matrix-Assisted Laser Desorption Ionization-Time-Of-Flight Mass Spectrometry In Comparison To Rpob Gene Sequencing For Species Identification Of Bloodstream Infection Staphylococcal Isolates. Clinical Microbiology And Infection. 2011;17(1):44-49.
[42]. Szarapińska-Kwaszewska J, Farkas Łi. Synthesis Of Siderophores By Strains Of Staphylococcus Cohnii Isolated From Various Environments. Polskie Towarzystwo Mikrobiologów The Polish Society Of Microbiologists. 2003;52(3):261-269.
[43]. Szewczyk Em.; Rozalska M. Staphylococcus Cohnii--Resident Of Hospital Environment: Cell-Surface Features And Resistance To Antibiotics. Acta Microbiologica Polonica. 2000;49(2):121-133.
[44]. Thorberg Bm. Coagulase-Negative Staphylococci In Bovine Sub-Clinical Mastitis. Department Of Biomedical Sciences And Veterinary Public Health, Swedish University Of Agricultural Sciences, 2008.
[45]. Viguier C, Arora S, Gilmartin N, Et Al. Mastitis Detection: Current Trends And Future Perspectives. Trends In Biotechnology. 2009;27(8):486-493.