RESUMO 45. SUSCEPTIBILITY OF MASTITIS -CAUSING PATHOGENS (Escherichia coli AND Staphylococcus aureus) TO ANTISEPTICS USED AS DIPPING

Maysa Serpa Gonçalves[1]; Marcilene Daniel Damasceno[1]; Alice Gonçalves dos Reis[1]; Bruna Henrique Pinto da Silva[1]; Giovanna Botelho Carneiro[1]; Alessandro de Sá Guimarães[2]; Elaine Maria Seles Dorneles[1]

[1]Universidade Federal de Lavras - Lavras - MG – Brasil
[2]Embrapa Gado de Leite - Juiz de Fora - MG - Brasil

INTRODUCTION

Mastitis is the most important and costly infectious disease in dairy farms. Bacteria is the main cause and treatment often requires antimicrobial drugs. Consequently, mastitis is the main source of antibiotic use in dairy farms, contributing to the emergence of antimicrobial-resistant strains. Aiming to prevent mastitis, many farms adopt teat dipping, disinfecting cow's teats with antiseptic solutions before and after the milking process. Although very efficient, it is hypothesized that dipping also creates selective pressure for the emergence of resistant bacteria, since microorganisms are constantly exposed to antiseptics. Resistance to antiseptics is a huge concern, since mechanisms are usually unspecific, conferring cross-resistance to several antimicrobial principles simultaneously, including antibiotics. Thus, this type of resistance can prejudice both prevention and treatment of bacterial diseases. However, despite susceptibility to antiseptics being widely assessed in other fields, such as hospitals and the food industry, it is still poorly studied in mastitis-causing pathogens. This study aimed to determine the susceptibility profile of S. aureus and E. coli isolated from bovine mastitis to antiseptics commonly used as dipping in dairy farms.

MATERIAL AND METHODS

Four hundred (400) *S. aureus* and 52 *E. coli* isolated from bovine mastitis cases were used to assess susceptibility to antiseptics. These strains are from four Brazilian states and were obtained between 1994 and 2016. The minimum inhibitory concentration (MIC) was obtained for six antiseptics: chlorhexidine, hydrogen peroxide, iodine, lactic acid, quaternary ammonium, and sodium hypochlorite. Reference concentrations for use in the field were obtained from a review conducted by the National Mastitis Council (NMC).

The minimum inhibitory concentration (MIC) for each antiseptic was obtained by the technique of microdilution in broth, adapting recommendations of the Clinical and Laboratory Standards Institute (CLSI) (manuals M100 and VET01S) for aerobic bacteria. Each strain was grown on Mueller Hinton agar and incubated at 37 ºC for 24 hours. Inoculums were prepared in 0.85% saline solution using the 0.5 McFarland. Each antiseptic was diluted in sterile Mueller Hinton broth to assess the maximum concentration tested (Tabela 1). Plates were incubated at 37 ºC, for 16-20h. As there are no established patterns for the interpretation of susceptibility tests carried out with antiseptics, we conducted five consecutive assays with the reference strains *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, to obtain MIC ranges for each of them. The reference strain with the most suitable MIC range for each antiseptic was chosen as a control for all the assays. Descriptive analyses of results and graphs were made using R software (version 4.4.1).

RESULTS AND DISCUSSION

MIC results of the six tested antiseptics are summarized in Table 1. In general, antiseptic concentrations recommended by NMC were sufficient to inhibit all strains, except for sodium hypochlorite (Table 1). For *E. coli*, 81% (42/52) of the strains required at least 1.25% sodium hypochlorite, more than twice the concentration used in the field (0.5%). Most *S. aureus* isolates were inhibited with lower concentrations, however, seven isolates [7/400 (1.75%)] had a MIC of 1.25%, suggesting an emergence of more resistant bacteria also in this species. In addition, a relationship was observed between the isolation year and the value of MIC, with more recent strains requiring higher

concentrations for being inhibited. This was observed not only for sodium hypochlorite but also for lactic acid (Figure 1).

These results suggest that continuous use of antiseptics in dairy farms can lead to significant selective pressure and the emergence of antiseptic-resistant strains, similar to what is observed for antibiotics. Sodium hypochlorite results are particularly alarming since this is an active principle used not only as an antiseptic but also as a disinfectant for cleaning milking installations in dairy farms. Additionally, this is a concern for public health, since both *E. coli* and *S. aureus* are zoonotic pathogens and livestock is one of the main sources of resistant strains for the human population.

Table 1. Minimum inhibitory concentration (MIC) values of antiseptic used as dipping for *E. coli* and *S. aureus* isolated from bovine mastitis in Brazil, 1994 – 2016.

Antiseptics	Recommended concentration* (%)	Concentration range (%)	MIC Average (%)		MIC Mode (%)		MIC50 (%)		MIC90 (%)	
			EC	SA	EC	SA	EC	SA	EC	SA
Chlorhexidine	350	0.002 - 1.4	0.002 (±0.000)	0.002 (±0.000)	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002	≤0.002
Hydrogen peroxide	640	0.002 - 1	0.003 (±0.002)	0.002 (±0.001)	≤0.002	≤0.002	≤0.002	≤0.002	4	≤0.002
Iodine	1000	0.002 - 1	0.014 (±0.004)	0.008 (±0.004)	16	8	16	8	16	16
Lactic acid	2640	0.021 - 10.56	0.190 (±0.082)	0.132 (±0.071)	165	82	165	82	330	165
Quaternary ammonium	500	0.004 - 2	0.019 (±0.017)	0.004 (±0.002)	16	≤0.004	16	≤0.004	31	≤0.004
Sodium hypochlorite	500	0.004 – 2.5	1.171 (±0.455)	0.392 (±0.216)	1250	310	1250	310	1250	620

*recommended by National Mastitis Council; MIC: minimum inhibitory concentration; SA: *S. aureus*; EC: *E. coli*.

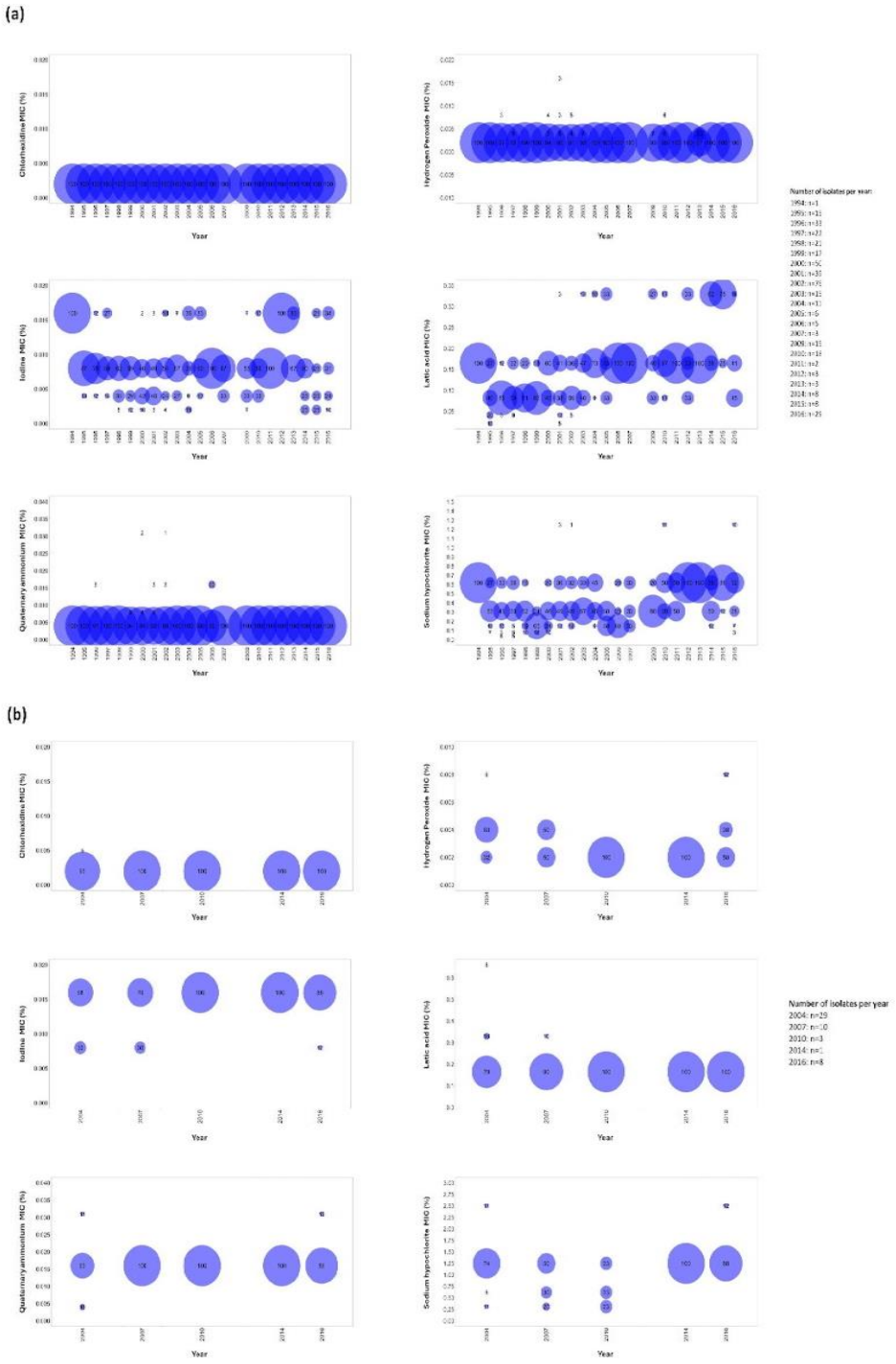

Figure 1. Percentage of strains of mastitis-causing *Staphylococcus aureus* (a) and *Escherichia coli* (b) inhibited in vitro with different concentrations of six antiseptics commonly used as dipping in dairy farms, according to year of isolation, 1994-2016, Brazil.

CONCLUSION

In conclusion, mastitis-causing S. aureus and E. coli were susceptible in vitro to chlorhexidine, hydrogen peroxide, iodine, lactic acid, and quaternary ammonium in the concentrations used in the field. For sodium hypochlorite, 81% of E. coli had a MIC higher than the recommended for use as dipping. These results are alarming for animal and human health and highlight the need for monitoring antiseptic resistance in mastitis-causing pathogens.

AGRADECIMENTOS

Fapemig, CNPq, CAPES.