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OVERVIEW: USE OF ANTIMICROBIALS IN FERMENTATION PROCESSES IN THE FUEL-ETHANOL INDUSTRY IN BRAZIL

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ABSTRACT

Bacterial contamination in fermentation processes for bioethanol production is one of the most important problems, resulting in significant fermentation losses in this industry. The main strategy to overcome this challenge in the industry is the use of a variety of antimicrobials, including antibiotics for human and animal use. This indiscriminate practice may intensify antimicrobial resistance (AMR) and result in multidrug-resistant (MDR) microorganisms. This study aimed to outline a current overview of antimicrobial use in sugarcane fuel ethanol fermentation in Brazil, addressing some industrial aspects and the environmental and human health consequences. Interviews with 10 Brazilian industrial units (2019/2020 harvest) revealed that all units monitor and control contamination, with the use of antibiotics being the most prevalent and frequent strategy. The most common antibiotics include monensin sodium, virginiamycin, penicillin, quinolones, and aminoglycosides. The frequency of use varies, with 50% of units using antibiotics continuously. Ninety percent of the units had never used alternative strategies to traditional antibiotics, and the only one that did reported low effectiveness. Therefore, considering what is already known: the continuous and unrestricted use of antibiotics increases the selective pressure for resistant or multidrug-resistant microorganisms, with worrying implications for human health (transfer of resistance genes) and the environment. Overall, these findings present a worrying scenario about contamination control in the bioethanol industry and emphasize the ecological and socioeconomic importance of further research into alternatives to antibiotics in bioethanol production processes, highlighting the imminent need for new technological solutions.

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Keywords: Antimicrobial use, Fuel ethanol fermentation, Bacterial contamination, Antibiotic resistance, Bioethanol industry, Multidrug-resistant bacteria

1. INTRODUCTION

Bacterial contamination in fermentation processes in the bioethanol industry is a very common problem and causes numerous losses not only to process yield but also indirect losses, since the presence of some contaminants contributes to triggering yeast flocculation and possible shutdowns with consequent losses.

Considering the nature of the industrial process and the large volumes of substrates processed, maintaining aseptic conditions is a particular challenge. For this reason, fermentation processes often operate under bacterial contamination. This is frequently considered one of the main drawbacks in the industrial ethanol production context. In addition to consuming sugars from feedstock sources (sugar cane juice or molasses) that would otherwise be converted to ethanol, some bacterial metabolites (such as lactic and acetic acids) may act negatively over the yeasts, affecting fermentation performance, resulting in reduced ethanol yield, promoting yeast cell flocculation and/or foaming, and low yeast yields (Narendranath et al., 1997; Bayrock & Ingledew, 2004). Bacterial-induced yeast flocculation may reduce the surface area of contact between the yeast and the medium (depending on the level of contamination) and usually impairs process efficiency. Besides, it demands changes in the process to improve and achieve an efficient centrifugation. Furthermore, an excessive foam formation increases costs due to the need for larger amounts of antifoams to reduce headspace. Therefore, the use of antibiotics to reduce contamination incurs additional costs, and their residual levels make dry yeast (a byproduct of the ethanol industry) unviable for human consumption or animal feed.

Various antimicrobials, including antibiotics used to treat infections that affect human and animal health, such as penicillin and virginiamycin, are frequently used to control bacterial contamination in fermentation processes for bioethanol production (Bischoff et al., 2007; Stroppa et al., 2000). The strategy for using these antimicrobials varies from one industrial unit to another. In some, the addition of antimicrobials to the fermentation process is continuous, while in others, antimicrobials are added in high doses according to bacterial contamination levels, which are constantly monitored. The common factor among contamination control strategies is the continuous use of antibiotics throughout virtually the entire harvest period.

Over the past two decades, the indiscriminate use of antibiotics to control bacteria has become common practice in various settings, extending beyond the use of these substances as antimicrobials to treat infections that affect human health. Historically, this unbalanced use of antibiotics, since the introduction of the first antimicrobial in 1937, has fueled a persistent increase in the spread of drug-resistant bacteria, widely known as antimicrobial resistance (AMR) (Davies

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& Davies, 2010). The consequences of AMR have implications for human health, including the emergence and increase in cases of multidrug-resistant microorganisms (MDR). Furthermore, O'Neill (2016) estimates that the rise of uncontrolled antimicrobial resistance will lead to 10 million preventable deaths per year by 2050. Antimicrobial resistance (AMR) by definition is the phenomenon in which infectious microorganisms, such as bacteria, have the ability to survive exposure to drugs that would normally inhibit their growth or kill them (O'Neill, 2016). The consequences of AMR have implications for human health, including the emergence and increase in cases of multidrug-resistant microorganisms (MDR). Furthermore, O'Neill (2016) estimates that the increase in uncontrolled antimicrobial resistance will lead to 10 million preventable deaths per year by 2050. On the other hand, Smith (2013) points out consequences that exceed the human cost when addressing economic factors due to the increase in AMR, since antimicrobial resistance results in higher health care costs and increased resource use (Alumran et al., 2014; Maragakis et al., 2008; McGowan, 2001). It is estimated that by 2050, if there are no restrictions, AMR will have impacted global production by US$100 trillion (O'Neill, 2016). From an evolutionary perspective, antimicrobial resistance is inevitable, since mutations and the ability to adapt to stressful situations and exposure to antimicrobials are inherent to bacteria (Fowler et al., 2014; Weber & Courvalin, 2005). However, knowing that the rate of AMR development is widely known, as well as facilitated by unnecessary and indiscriminate use, actions can be taken to delay and contain the development of resistance (O'Neill, 2016; Buke et al., 2005). Therefore, the indiscriminate use of antibiotics in industrial processes can undoubtedly harm both human health and the environment, as antibiotics have the potential to leave residues, both related to the original antimicrobial and its metabolites and/or conjugates (Isidori et al., 2005; Islam et al., 2016; Peters et al., 2009). Thus, to outline a current overview of the Brazilian scenario of antimicrobial use in fermentation processes in bioethanol production, this study addresses the practices of antimicrobial use in the fermentation processes of Brazilian sugarcane industrial units. This practice is related to both industrial aspects (such as ethanol yield and productivity losses) and the main environmental challenges and consequences for human health. Furthermore, the discussion of the current paradigm for antimicrobial use not only provides theoretical elements but also presents data collected from interviews with ten Brazilian industrial units regarding the use of antimicrobials to control bacterial contamination in fermentation processes during the 2019/2020 harvest season periods.

2. MATERIAL AND METHODS

Interviews were conducted with 10 Brazilian industrial units producing fuel ethanol during the 2019/2020 harvest season. Nine of these industrial units are located in the state of São Paulo and one in the state of Mato Grosso do Sul. All use sugarcane as a feedstock for fuel ethanol production in their fermentation processes. The interviews were conducted in 2019, and the questions

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considered the practices adopted as a reference for the 2019/2020 harvest season and, in the case of questions 9 and 10, considered the practices adopted for all harvest seasons, including previous harvests and the 2019/2020 harvest season. The interviews were structured around a verbal questionnaire that listed ten questions addressing bacterial contamination control strategies used in fermentation processes. The purpose was to identify the strategies used, as well as the most commonly used antimicrobials and their frequency of use/application. The questions used in the questionnaire are listed in Table 1.

TABLE 1: Questions used in the interview with industrial units to evaluate bacterial contamination control strategies used in fermentation processes

#	**Questions used in the interview with industrial units**
Q1	What is the location of the industrial unit?
Q2	Does the industrial unit monitor bacterial contamination?
Q3	Does the industrial unit carry out bacterial contamination control?
Q4	If you use bacterial contamination control strategies, which one(s) do you use?
Q5	Among the contamination control strategies used, which is the most frequently used?
Q6	Do you use antibiotics to control bacterial contamination?
Q7	Which antibiotics were used?
Q8	How often are the antibiotic(s) used?
Q9	Have you ever used any alternative or natural strategies to control contamination to replace the use of antibiotics?
Q10	If yes to question 9, what is your perception of the results obtained?

3. RESULTS AND DISCUSSION

The collected data from the interviews are described below, with the identities of the industrial units being preserved for ethical and confidentiality reasons, as the information was provided for this work collaboratively. Therefore, the nomenclature adopted for the industrial units will consist only of sequential letters. Both the objective responses to questions 2 through 6 and question 9 (Q9), as well as the subjective responses to questions 7, 8, and 10, will be discussed below.

Considering the units evaluated, 100% reported monitoring bacterial contamination and using contamination control strategies. Among the contamination control strategies, four were identified mainly: 1) use of antibiotics; 2) addition of acid to yeast or acid treatment; 3) use of chlorine dioxide; and 4) use of hop extract (Table 2).

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Table 2: Bacterial contamination control strategies used in fermentation processes in Brazilian industrial units different states of Brazil. N=10

Industrial	State of	Strategies used in fermentation processes			
Unit	Brazil	ANT	AAC	DCL	EXL
A	SP				
B	SP				
C	SP				
D	SP				
E	MS				
F	SP				
G	SP				
H	SP				
I	SP				
J	SP				

The use of antibiotics as a contamination control strategy was prevalent and reported by 100% of the industrial units, followed by 30% of the units that also used chlorine dioxide. Another 30% of the industrial units used acid addition as a containment strategy in addition to antibiotics, and only 1 unit, or 10%, mentioned the use of hop extract to control bacterial contaminants (Figure 1). Regarding predominant use, 100% of the units indicated the use of antibiotics as the most frequent practice for controlling bacterial contamination in the fermentation process. It is also important to highlight that 60% of industrial units adopt combined contamination control practices, as can be observed in Table 2.

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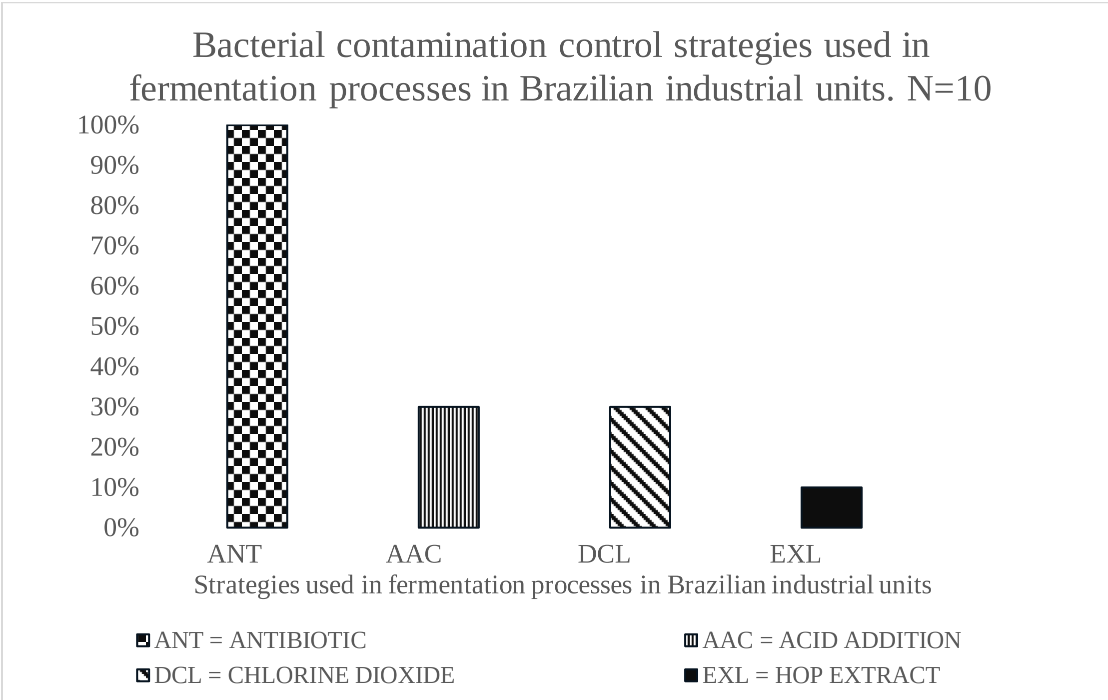

Figure 1: Proportion of contamination control strategies used in fermentation processes in Brazilian industrial units. N=10

Regarding the antibiotics used by the industrial units, 100% reported using sodium monensin, 30% use it in combination with virginiamycin, and another 20% stated that they use, in addition to these two antibiotics, other types of antibiotics such as penicillin, quinolones, and aminoglycosides (gentamicin, neomycin), accounting for the use of more than five types of antibiotics intercalated. When asked about the frequency of use and/or application of antibiotics in the fermentation process, there was no single pattern of response, with responses varying widely. The resulting profiles for this question are as follows: 50% use antibiotics continuously and add antibiotics constantly; 10% add antibiotics hourly to control contamination, regardless of contamination levels; 20% apply antibiotics according to contamination levels, considering bacterial count data to keep them below 10^6; 10% stated that they use antibiotics only when contamination occurs, but that this situation persists for more than 95% of the harvest, thus using several antibiotics alternately; and 10% stated that they use antibiotics sporadically, as they continuously use chlorine dioxide in the process (Figure 2).

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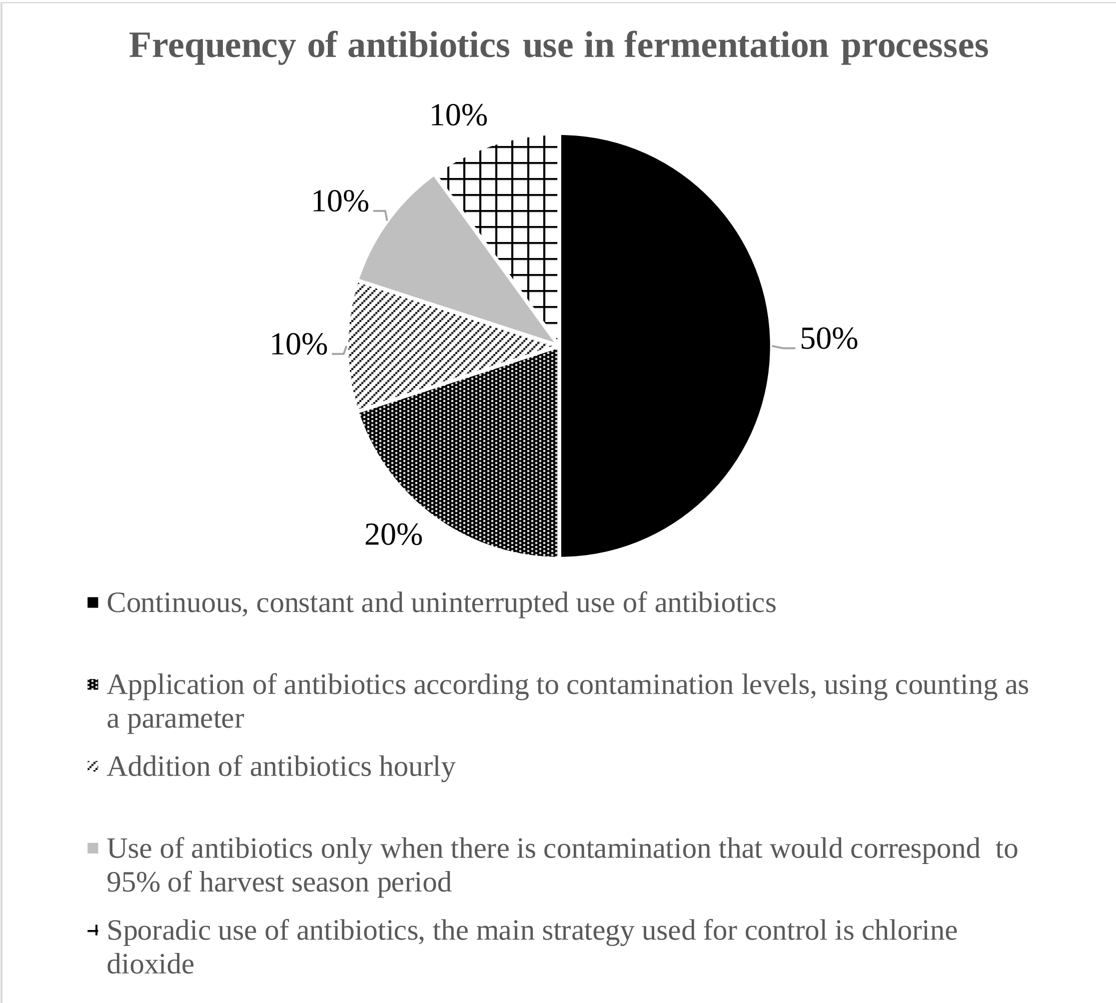

Figure 2: Frequency of use/addition of antibiotics in fermentation processes for fuel etanol production in Brazilian industrial units. N=10.

Regarding the experience of industrial units in using alternative or natural strategies to control contamination as a substitute for antibiotics, 90% of the industrial units reported never having used other alternative strategies with natural products, and only 10% reported having used them. However, this unit highlighted limitations related to the low effectiveness of maintaining this type of application practice. The results presented through data collected in interviews with the industrial units demonstrate the continuous and unrestricted use of antibiotics in fermentation processes, without defined criteria. This contamination control strategy increases selection pressure and may also increase the incidence of microorganisms resistant or multi-resistant to antibiotics.

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Several authors have demonstrated high levels of antibiotic resistance in microorganisms from environments with high antibiotic exposure (Dec et al., 2017). Furthermore, the ubiquity of LAB in nature, combined with the fact that Gram-positive bacteria, especially those of the genus *Lactobacillus sp.*, have been considered reservoirs of antibiotic resistance genes (Murphree et al., 2014; Saaed et al., 2014), reinforce concerns about the transfer of resistance genes to pathogenic microorganisms, causing environmental problems and consequences for human health that this indiscriminate use of antibiotics in the industrial context for controlling bacterial contamination in fermentation processes can bring. Therefore, several authors have recommended the prudent use of antibiotics and the reduction of their excessive use outside of medical and veterinary practice (Dec et al., 2017; Murphree et al., 2014; Saaed et al., 2014).

4. CONCLUSION

Antibiotics, used to reduce contamination, generally increase operational costs in the industry, and their residual levels make dry yeast (a byproduct of the ethanol industry) unsuitable for commercialization (for human consumption or animal feed). Therefore, the results presented in this study and the information in the literature on the selection of antibiotic-resistant microorganisms point to a worrying outlook in the Brazilian industrial and environmental landscape, considering the use of antimicrobials in fermentation processes. Traditionally, ethanol production requires efficient solutions to control bacterial contamination in the processes, as well as the imminent need for new technological solutions capable of helping to reduce the excessive and widespread use of antibiotics in contexts beyond their application in human or animal health. Therefore, more research on this topic is needed, including the search for alternative solutions to the use of antibiotics to control contamination in fermentation processes for bioethanol production.

REFERENCES

[1]. Alumran, A., Hou, X. Y., Sun, J., Yousef, A. A., & Hurst, C. (2014). Assessing the construct validity and reliability of the parental perception on antibiotics (PAPA) scales. *BMC Public Health*, *14*(1), 73.

[2]. Bayrock, D. P., & Ingledew, W. M. (2004). Inhibition of yeast by lactic acid bacteria in continuous culture: nutrient depletion and/or acid toxicity? *Journal of Industrial Microbiology & Biotechnology*, *31*(8), 362–368.

[3]. Bischoff, K. M., Skinner-Nemec, K. A., & Leathers, T. D. (2007). Antimicrobial susceptibility of *Lactobacillus* species isolated from commercial ethanol plants. *Journal of Industrial Microbiology & Biotechnology*, *34*, 739–744.

[4]. Buke, C., Hosgor-Limoncu, M., Ermertcan, S., Cicekliogl, M., Tuncel, M., Köse, T., & Eren, S. (2005). Irrational use of antibiotics among university students. *Journal of Infection*, *51*(2), 135–139.

 Copyright © 2025 by the authors. Licensed under CC BY-NC-SA 4.0

[5]. Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews*, *74*(3), 417–433.

[6]. Dec, M., Urban-Chmiel, R., Stępień-Pyśniak, D., & Wernicki, A. (2017). Assessment of antibiotic susceptibility in *Lactobacillus* isolates from chickens. *Gut Pathogens*, *9*, 54. https://doi.org/10.1186/s13099-017-0203-z

[7]. Fowler, T., Walker, D., & Davies, S. C. (2014). The risk/benefit of predicting a post-antibiotic era: is the alarm working? *Annals of the New York Academy of Sciences*, *1323*(1), 1–10.

[8]. Isidori, M., Lavorgna, M., & Nardelli, A. (2005). Toxic and genotoxic evaluation of six antibiotics on non-target organisms. *Science of the Total Environment*, *346*(1-3), 87–98.

[9]. Islam, A., Saifuddin, A. K. M., Faruq, A. A., Islam, S., Shano, S., Alam, M., & Hassan, M. M. (2016). Antimicrobial residues in tissues and eggs of laying hens at Chittagong, Bangladesh. *International Journal of One Health*, *2*(2), 75–80.

[10]. Maragakis, L. L., Perencevich, E. N., & Cosgrove, S. E. (2008). Clinical and economic burden of antimicrobial resistance. *Expert Review of Anti-infective Therapy*, *6*(5), 751–763.

[11]. McGowan, J. E., Jr. (2001). Economic impact of antimicrobial resistance. *Emerging Infectious Diseases*, *7*(2), 286–292.

[12]. Murphree, C. A., Heist, P. E., & Moe, L. A. (2014). Antibiotic resistance among cultured bacterial isolates from bioethanol fermentation facilities across the United States. *Current Microbiology*. https://doi.org/10.1007/s00284-014-0583-y

[13]. Narendranath, N. V., Hynes, S. H., Thomas, K. C., & Ingledew, W. M. (1997). Effects of lactobacilli on yeast-catalyzed ethanol fermentations. *Applied and Environmental Microbiology*, *63*(11), 4158–4163.

[14]. O'Neill, J. (2016). *The review on antimicrobial resistance: tackling drug-resistant infections globally: final report and recommendations*. The Review on Antimicrobial Resistance.https://amrreview.org/sites/default/files/160518_Final%20paper_with%20cover.pdf

[15]. Peters, R. J. B., Block, Y. J. C., Rutger, S. P., Stolker, A. A. M., & Nielen, M. W. F. (2009). Multiresidue screening of veterinary drugs in egg, fish and meat using high-resolution liquid chromatography accurate mass time-of-flight mass spectrometry. *Journal of Chromatography A*, *1216*(46), 8206–8216.

[16]. Saaed, R. M., Elyas, Y. Y. A., Yousif, N. M., Eltayeb, M. M., & Ahmed, I. S. M. (2014). Incidence of antibiotic resistance of lactic acid bacteria (LAB) isolated from various Sudanese fermented foods. *Journal of Food and Nutritional Disorders, 3*(6). https://doi.org/10.4172/2324-9323.1000161

[17]. Smith, R., & Coast, J. (2013). The true cost of antimicrobial resistance. *BMJ*, *346*, f1493.

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[18]. Stroppa, C. T., Andrietta, M. G. S., Andrietta, S. R., Steckelberg, C., & Serra, G. E. (2000). Use of penicillin and monensin to control bacterial contamination of Brazilian alcohol fermentations. *International Sugar Journal*, *102*, 78–82.

[19]. Weber, J. T., & Courvalin, P. (2005). An emptying quiver: Antimicrobial drugs and resistance. *Emerging Infectious Diseases*, *11*(6), 791.

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