Revista Eletrônica
Acervo Saúde
Electronic Journal Collection Health ISSN 2178-2091

Phenotypic characterization of bacterial isolates resistant to sodium hypochlorite (0.025%) obtained from lettuce sold in the municipality of Ananindeua, state of Pará, Brazil

Caracterização fenotípica de isolados bacterianos resistentes ao hipoclorito de sódio (0,025%) obtidos de alfaces comercializados no município de Ananindeua, estado do Pará, Brasil

Caracterización fenotípica de aislados bacterianos resistentes al hipoclorito de sodio (0,025%) obtenidos de lechuga comercializada en el municipio de Ananindeua, estado de Pará, Brasil

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ABSTRACT

Objective: The study aimed to perform a phenotypic characterization of bacterial isolates resistant to 0.025% sodium hypochlorite obtained from lettuce samples commercialized in the metropolitan region of Belém, Brazil, assessing their resistance and antimicrobial susceptibility profiles. **Methods:** Lettuce samples were collected from supermarkets and open markets. Bacterial counts were conducted on Plate Count Agar supplemented with 0.025% sodium hypochlorite. Isolates were biochemically identified, and the Minimum Inhibitory Concentration (MIC) was determined. Antimicrobial susceptibility tests using disk diffusion assessed resistance to antibiotics. **Results:** Although sodium hypochlorite reduced 99% of bacterial colonies, 84 resistant isolates were identified, primarily Gram-positive Bacilli (45%) and Enterobacteriaceae (31%), including Escherichia coli and Salmonella spp. The mean MIC was 0.1%, and three multidrug-resistant profiles were observed among Enterobacteriaceae, displaying resistance to amoxicillin and other antibiotics. A significant inverse correlation was found between the MIC and inhibition halos formed by amoxicillin ($r = -0.24$; $p = 0.0423$). **Conclusion:** Sodium hypochlorite at the recommended concentration is effective, but the detection of resistant and multidrug-resistant strains raises concerns about bacterial adaptation. Continuous monitoring is necessary to update sanitization protocols and safeguard public health.

Keywords: Biocides, Microbial resistance, Microbiological quality, Bacterial isolates.

RESUMO

Objetivo: Caracterizar fenotipicamente isolados bacterianos resistentes a 0,025% de hipoclorito de sódio obtidos de amostras de alface comercializadas na região metropolitana de Belém, Brasil, e avaliar seus perfis de resistência e suscetibilidade antimicrobiana. **Métodos:** Amostras de alface foram coletadas em supermercados e mercados populares. Contagens bacterianas foram realizadas em meio de Ágar Plate Count suplementado com 0,025% de hipoclorito. Isolados foram identificados bioquimicamente, e a Concentração

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SUBMETIDO EM: 12/2024 | ACEITO EM: 1/2025 | PUBLICADO EM: 3/2025

Inibitória Mínima (MIC) foi determinada. Testes de sensibilidade antimicrobiana com discos de difusão avaliaram a resistência a antibióticos. **Resultados:** Embora o hipoclorito tenha reduzido 99% das colônias bacterianas, 84 isolados resistentes foram identificados, principalmente Bacilos Gram-positivos (45%) e Enterobacteriaceae (31%), incluindo Escherichia coli e Salmonella spp. A MIC média foi 0,1%, e três perfis multirresistentes foram observados entre Enterobacteriaceae, com resistência a amoxicilina e outros antibióticos. Uma correlação inversa significativa foi encontrada entre a MIC e os halos formados por amoxicilina (r = -0,24; p=0,0423). **Conclusão:** O hipoclorito de sódio na concentração recomendada é eficaz, mas a detecção de cepas resistentes e multirresistentes alerta para o risco de adaptação bacteriana. Monitoramento contínuo é necessário para atualizar protocolos de sanitização e proteger a saúde pública.

Palavras-chave: Biocidas, Resistência microbiana**,** Qualidade microbiológica, Isolados bacterianos.

RESUMEN

Objetivo: Caracterizar fenotípicamente aislados bacterianos resistentes al 0,025% de hipoclorito de sodio obtenidos de muestras de lechuga comercializadas en la región metropolitana de Belém, Brasil, y evaluar sus perfiles de resistencia y susceptibilidad antimicrobiana. **Métodos:** Se recogieron muestras de lechuga en supermercados y mercados populares. Se realizaron recuentos bacterianos utilizando Agar Plate Count suplementado con 0,025% de hipoclorito de sodio. Los aislados fueron identificados bioquímicamente y se determinó la Concentración Inhibitoria Mínima (MIC). Las pruebas de susceptibilidad antimicrobiana mediante difusión en disco evaluaron la resistencia a los antibióticos. **Resultados:** Aunque el hipoclorito de sodio redujo el 99% de las colonias bacterianas, se identificaron 84 aislados resistentes, principalmente Bacilos Gram-positivos (45%) y Enterobacteriaceae (31%), incluidos Escherichia coli y Salmonella spp. La MIC media fue de 0,1%, y se observaron tres perfiles multirresistentes entre Enterobacteriaceae, con resistencia a la amoxicilina y otros antibióticos. Se encontró una correlación inversa significativa entre la MIC y los halos formados por la amoxicilina (r = -0,24; p=0,0423). **Conclusión:** El hipoclorito de sodio en la concentración recomendada es eficaz, pero la detección de cepas resistentes y multirresistentes plantea preocupaciones sobre la adaptación bacteriana. Es necesario un monitoreo continuo para actualizar los protocolos de sanitización y proteger la salud pública.

Palabras clave: Biocidas, Resistencia microbiana, Calidad microbiológica, Aislados bacterianos.

INTRODUCTION

In recent decades, there has been an exponential growth in the world population, resulting in an increasing demand for food. This phenomenon has driven the increase in food production, especially concerning agricultural products, which have gained a larger share in the global market (SANTOS DAC e GARCIA PPC, 2018).

In this context, ensuring the microbiological safety of these foods is a concern for both developed and developing countries. Consequently, hygienic-sanitary protocols and monitoring projects for the food production chain have been developed in each country to preserve the quality and maintain the microbiological standards of products aiming to combat the spread of Foodborne Illnesses (FBIs) (AVILA MO, et al., 2016).

FBIs pose a global public health problem, causing thousands of deaths annually and resulting in billions of dollars in costs for the prevention and treatment of pathologies associated with inadequately regulated food production (PAHO, 2019). Therefore, the World Health Organization (WHO) emphasizes that food hygiene is crucial in combating FBIs, along with monitoring bacteria resistant to antimicrobials and especially to biocides used in food sanitation (JÚNIOR AAN, et al., 2023).

Despite the significant burden on global health, research on cases related to FBIs in the northern region of the country is still limited (DUARTE GR, et al., 2023). Among the cities in the Amazon region is Belém, the capital which has a low Human Development Index (HDI), facing various issues such as lack of infrastructure and basic sanitation, consequently directly affecting the human and food health of the population (LIMA JS, et al., 2018).

Therefore, current food sanitation methods make use of biocides, chemical compounds primarily intended to eliminate pathogenic microorganisms, thus assisting in microbial control of fruits, vegetables, and other products in the domestic environment (JÚNIOR AAN, et al., 2023).

However, biocides are often used indiscriminately and incorrectly by society today. This irrational use results in selective pressure on the microbial community, favoring the presence of bacteria carrying resistance genes to these antimicrobials, thereby directly impacting microbial control and food safety (ONU, 2019).

Legislation aimed at the correct use of biocides in Brazil has been established by ANVISA (National Health Surveillance Agency) and the Ministry of Health since the 2000, establishing manuals for the proper use of biocides, advocating for Minimum Inhibitory Concentrations (MIC) of each product and for each type of sanitation (MINISTÉRIO DA SAÚDE, 2019). Sodium hypochlorite is the most effective bactericidal sanitizer for vegetables, a strong inducer of cellular biocompatibility (ESTRELA C, et al., 2002). This sanitizer is commercially available in concentrations of 2 to 2.5%, with the label prescribing a dilution of 1 tablespoon per 1 liter of water, ideal for effective sanitation (CFQ, 2020).

Despite the presence of protocols and guidelines, there is a need for continuous development of studies and monitoring regarding resistance to these biocides. New bacterial strains emerge annually, necessitating the monitoring of the effectiveness of current protocols to reduce food contamination and consequently FBIs (AVILA MO, et al., 2016).

Hence, there is a need for an expansion of studies and research aimed at monitoring the susceptibility of bacteria to current methods used for food disinfection, especially vegetables like lettuce, which due to the method in which they are cultivated, have high rates of transmission of bacterial diseases caused by different species (BERALDO RM, 2010). Therefore, the objective of this study is to perform phenotypic characterization of bacterial isolates resistant to the biocide (sodium hypochlorite 0.025%), obtained from lettuce samples (*Lactuca sativa* var. *Crispa*) commercialized in the metropolitan region of Belém, Brazil.

METHODS

Obtaining Samples

The present study was conducted in the municipality of Ananindeua, located in the Metropolitan Region of Belém, Pará state, Brazil. This area is an important hub for the commercialization of vegetables in the Amazon region, characterized by high demand for agricultural products in both formal and informal markets. However, the region faces significant challenges related to infrastructure and basic sanitation, factors that may contribute to the microbiological contamination of food.

A total of 13 samples were collected from supermarkets and 13 from open markets, with 6 used to evaluate and control the efficiency of sodium hypochlorite and 20 for phenotypic characterization. The samples were stored in plastic bags provided by the establishment and transported in properly sanitized insulated boxes. It is emphasized that the sample analysis was performed within 2 hours after collection.

Quantification Of Mesophilic Bacteria Resistant To Sodium Hypochlorite

The quantification of mesophilic bacteria was carried out using the Spread plate method (COSTA AJM, 2018). In a previously sterilized environment, 25 grams of each sample were weighed on sterile aluminum foil, with the aid of a precision analytical balance (Bel Engineering®) and a Bunsen burner. Subsequently, the samples were placed in 250 ml of sterile 0.85% saline solution and manually homogenized for 10 minutes.

A sterile glass pipette was used to transfer 1 ml of the homogenized solution to a tube containing 9 ml of sterile 0.85% saline solution, for a serial dilution. After homogenization, 1 ml of the dilution was transferred to another tube with 9 ml of sterile 0.85% saline solution to obtain a 10^{-2} concentration of the sample. The procedure was repeated to obtain a 10^{-3} concentration. It is important to note that a pilot test of the procedure was conducted, and the 10^{-3} concentration was deemed ideal for the subsequent step.

Subsequently, 100 µL of the 10^{-3} dilution of each sample was inoculated onto plates containing Agar Plate Count (Kasvi®) with and without supplementation of 0.025% sodium hypochlorite, as recommended by ANVISA. This culture was performed in triplicate, both in the presence and absence of sodium hypochlorite. Inoculation was performed using the spread plate technique with the aid of a sterile Drigalski loop. Afterward, the plates were incubated at 36°C for 48 hours in a microbiological incubator (SolidSteel®).

At the end of the incubation period, the Colony Forming Units (CFUs) on the plates with and without hypochlorite were counted using the Colony Counter® application.

Isolation And Biochemical Identification

For each lettuce sample, 25 grams were weighed on sterile aluminum foil using a precision analytical balance (Bel Engineering®). Subsequently, 250 ml of 0.85% saline solution was added and manually homogenized for 10 minutes. Next, 10 ml of this solution was transferred to 250 ml of Nutrient Broth (Kasvi®), supplemented with 0.025% sodium hypochlorite. The culture was then incubated at 36°C for 24 hours. Following this, isolation was performed on plates of Nutrient Agar supplemented with 0.025% Hypochlorite. The plates were incubated at 36°C for 48 hours.

The Colony Forming Units (CFUs) formed were transferred to tubes containing slanted Nutrient Agar (Kasvi®) and incubated at 36°C for 24 hours. All incubations were carried out in a microbiological incubator (SolidSteel®).

Subsequently, each isolate was subjected to Gram staining, following the guidelines recommended by the Ministry of Health (MINISTÉRIO DA SAÚDE, 2001). The morphology and arrangement of cells were evaluated to form a phenotypic profile of the isolates.

Gram-negative isolates underwent a biochemical series for the identification of strains of the Enterobacteriaceae family. The biochemical series included the following tests: Triple Sugar Iron (TSI), Citrate Agar, Lysine Agar, Motility Agar, Indole Agar, and Urea Broth. Identification was performed following the guidelines of Koneman (2006) and ANVISA (2015).

Determination Of Minimum Inhibitory Concentration (Mic).

Following the ANVISA protocols (ANVISA, 2001), the microdilution method in plates was performed to determine the Minimum Inhibitory Concentration (MIC) to sodium hypochlorite. This serial dilution was carried out in Mueller-Hinton broth (Kasvi®) at the following concentrations: 0.025%; 0.05%; 0.075%; 0.1%; 0.125%, 0.15%; 0.175%; 0.2%.

After the antimicrobial dilutions were completed, a suspension of each isolate previously prepared at a 0.5 McFarland turbidity scale was inoculated into the serial dilution. Subsequently, it was incubated at 37°C for approximately 24 hours in a bacteriological incubator. Afterward, the presence or absence of turbidity was observed according to the concentration of sodium hypochlorite applied. The lowest concentration that showed no turbidity will be considered as the Minimum Inhibitory Concentration (MIC).

Diffusion Disk Antimicrobial Sensitivity Test

The evaluation of antimicrobial sensitivity by disk diffusion was conducted following the protocols provided by the Brazilian Committee on Antimicrobial Susceptibility Testing, recommended by ANVISA and the Ministry of Health since Decree No. 64 of December 11, 2018. A suspension of each isolate was prepared in sterile 0.85% saline solution at a 0.5 McFarland turbidity scale. Subsequently, seeding was performed on Mueller-Hinton Agar (Laborclin/Pinhais-PR) using a sterile swab. Then, diffusion disks (Laborclin/Pinhais-PR) containing the tested antibiotics were added. The following antibiotics were tested: Amoxicillin (20 µg); Azithromycin (15 µg); Tetracycline (30 µg); and Ciprofloxacin (5 µg).

After completing this process, the plates were placed in a bacteriological incubator at 37°C for 24 hours, and the halo readings were performed in accordance with the BRCAST standard (BRCAST, 2021).

Data Analysis

After data collection, the data were organized into spreadsheets using the Excel program (Office 360 Package). A descriptive statistical analysis was performed, including the creation of tables (with absolute and relative frequencies) and a Box Plot graph (for quartile measures, median, variance, and mean).

Inductive analysis was conducted using the BioEstat program, employing the Pearson correlation test (r) to analyze the correlation between the variable "Minimum Inhibitory Concentration to Sodium Hypochlorite" and the diameters observed in the halos formed in the disk diffusion test of each antibiotic. Only p-values less than 0.05 will be considered statistically significant, while those equal to or greater than 0.05 will be considered borderline and statistically invalid.

RESULTS

The efficiency of disinfection with the biocide (Sodium Hypochlorite) following the recommendations of ANVISA was evaluated. The concentration of mesophilic bacteria (CFU/g) was analyzed in the presence and absence of this biocide (**Table 1).** An average reduction of 99% in the number of colonies was observed in the supplemented media, reinforcing the efficacy of Sodium Hypochlorite in food disinfection.

Table 1 - Concentration of mesophilic bacteria (CFU/g) from lettuce samples, according to supplementation with 0.025% Sodium Hypochlorite.

Supplementation of Plate Count Agar	Number of Samples	**Quantification of Mesophilic Bacteria (UFC.g-1)**	
		Average Concentration	Median Concentration
Absence of sodium hypochlorite (0,025%)	6	279	100
Supplemented with Hypochlorite sódio (0,025%)	6	>25	>25

Source: Sousa GSM, et al., 2025.

Despite the proven efficacy of the recommended biocide concentration, a total of 84 isolates resistant to 0.025% Sodium Hypochlorite were obtained (**Table 2**). It is noteworthy that these isolates were obtained from samples marketed in both supermarkets and open-air markets.

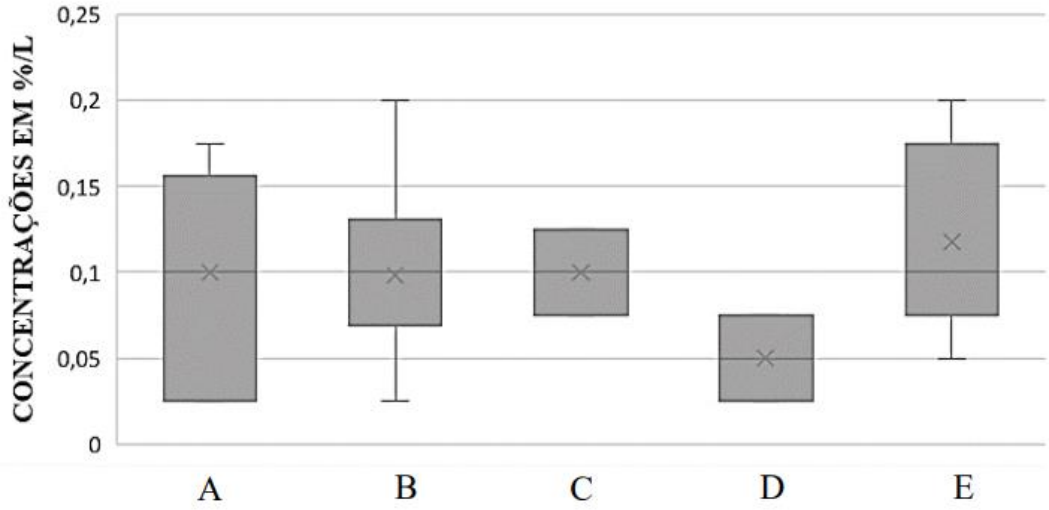
Table 2 - Biochemical identification of bacterial isolates resistant to 0.025% Sodium Hypochlorite, obtained from lettuce samples marketed in the Metropolitan Region of Belém.

Bacterial Group	**n (%)**
Enterobacteriaceae	26 (31%)
Escherichia coli	9 (12%)
Klebsiella pneumoniae	2 (2%)
Klebsiella aerogenes	7 (9%)
Klebsiella oxytoca	1 (1%)
Proteus vulgaris	1 (1%)
Proteus morabilis	1 (1%)
Pantoea aglomerans	2 (2%)
Serratia mascescens	1 (1%)
Salmonella sp.	2 (2%)
Bacilo Gram-negativo no Enterobacteriaceae	2 (2%)
Bacilo Gram positive	38 (45%)
Cocos Gram positive	15 (18%)
Cocos Gram negativos	3 (4%)
Total	84 (100%)

Source: Sousa GSM, et al., 2025.

Among the 84 isolates obtained, the highest frequency was observed for Gram-positive Bacilli (45%) and isolates from the Enterobacteriaceae family (31%). It is important to note that Escherichia coli was the most frequent species among the isolates of this family (35%), representing 12% of the total isolates obtained. After biochemical identification, the Minimum Inhibitory Concentration (MIC) of the identified strains was evaluated (**Figure 1**).

Figure 1 - Box plot (Quartile, median, and mean) of the Minimum Inhibitory Concentrations (MIC) observed in the identified bacterial groups: A - Gram-positive Bacilli; B - Enterobacteriaceae; C - Gram-negative Bacilli (Non-Enterobacteriaceae); D - Cocci.



Source: Sousa GSM, et al., 2025.

The mean MIC obtained for all strains was approximately 0.1% / L. The group of Gram-positive Cocci showed the highest median (0.125% / L), while Gram-negative Cocci showed the lowest median (0.05% / L). Regarding Enterobacteriaceae, a lower variance was observed in the MIC values obtained in this group.

The sensitivity profile to antibiotics of the isolates was determined after the MIC tests. In the Enterobacteriaceae group, seven different phenotypic profiles were defined (**Table 3**), including three multidrug-resistant profiles (C, D, and E).

The most frequent profile was A, among the identified phenotypes, showing sensitivity to all tested antimicrobials. It is noteworthy that profile D was the most frequent among multidrug-resistant isolates, showing resistance to Amoxicillin, Azithromycin, and Tetracycline. Among the identified phenotypes, it is emphasized that all observed profiles are sensitive to Ciprofloxacin.

Table 3 - Phenotypic profile of Enterobacteriaceae strains resistant to 0.025% Sodium Hypochlorite determined through antibiotic sensitivity testing (Disk diffusion).

		Antimicrobial			
Phenotype	**Isolated**	**Amoxicillin (20 µg)**	**Azithromycin (15 µg)**	**Tetracycline (30 µg)**	**Ciprofloxacin (5 µg)**
A	**12**	Sensitive	Sensitive	Sensitive	Sensitive
B	**6**	Resistant	Sensitive	Sensitive	Sensitive
C	**1**	Resistant	Resistant	Sensitive	Sensitive
D	**3**	Resistant	Resistant	Resistant	Sensitive
E	**2**	Resistant	Sensitive	Resistant	Sensitive
F	**1**	Sensitive	Sensitive	Resistant	Sensitive
G	**1**	Sensitive	Resistant	Sensitive	Sensitive

Source: Sousa GSM, et al., 2025.

A Pearson correlation test was performed (**Table 4**). Thus, according to the r and p values found, a significant inverse correlation was obtained between the halos of amoxicillin and the MIC levels. Among the halos of azithromycin, ciprofloxacin, and tetracycline, a non-significant correlation was observed with respect to MIC.

Table 1 - Pearson correlation coefficient (r) between the Minimum Inhibitory Concentration (MIC) of Sodium Hypochlorite and the Halos of the antimicrobial sensitivity test.

	Halos of Amoxicillin	Halos of Azithromycin	Halos of Ciprofloxacin	Halos of Tetracycline
CIM hypochlorite	r = - 0,24;	r = - 0,12;	r= 0,06;	r = - 0,22;
	p= 0,0423	p= 0, 443	p= 0,447	p= 0,543

Source: Sousa GSM, et al., 2025.

DISCUSSION

Sodium hypochlorite is a sanitizer commonly recommended for the hygiene of vegetables by health organizations. This sanitizer has a hydrophilic character and easily binds to protein channels and fatty acids, initiating saponification reactions. These reactions dissolve the organic membrane, releasing chlorine ions into the intracellular medium, thereby increasing its pH. Additionally, there is an irreversible binding to the amino group of amino acids, forming chloramines, which destabilize cellular metabolism by altering the pH gradient and inactivating essential enzyme components of bacterial physiology (ESTRELA C, et al., 2002).

In this context, this biocide proved effective for bacteriological control (**Table 1**), especially at the concentration recommended by ANVISA (0.025%/L). Thus, it is perceived that the molecular constitution and the cascade of harmful effects caused by hypochlorite on bacteria are decisive factors for its broad bactericidal spectrum. This fact may be related to non-generalist mechanisms of resistance to hypochlorite (such as alterations in the binding site), as they are essentially inefficient against the broad spectrum of reactions caused (ÉVORA BHRS, 2019).

However, over the past decades, there has been a greater incidence of multidrug-resistant microorganisms due to indiscriminate and incorrect use of antimicrobials, resulting in increasingly lower susceptibility levels to various antibiotics and biocides, such as sodium hypochlorite (CARVALHO et al., 2021). In this present study, bacterial strains resistant to the recommended CIM levels for vegetable sanitation were isolated, mostly Gram-positive Bacilli (**Table 2**), which are recognized as a pertinent bacterial group in various environments, such as soil (CAUMO et al., 2010).

The second largest isolated bacterial group was Enterobacteriaceae (**Table 2**). This bacterial family is mainly present in the gastrointestinal tract and is known to include species of great clinical relevance, with high levels of pathogenicity, virulence, and multidrug resistance, both to antibiotics and biocides, being considered the main causes of opportunistic infections related to health care (PÉREZ GUERRERO et al., 2014). Among the isolated species of this family, the most frequent was *Escherichia coli* (**Table 2**), a bacterium present in the human intestinal microbiota and an important bioindicator of fecal contamination (NORONHA TH et al., 2019).

Among the Enterobacteriaceae isolates, strains of *Salmonella* spp. (**Table 2**) also stand out, known for causing severe cases of foodborne illness. The presence of strains of this species reinforces the possibility of fecal contamination of water or soil, emphasizing the need for sanitary hygiene to maintain consumer safety (SANTANA EHWD, et al., 2010). The group of Gram-positive Cocci was the third most frequent (**Table 2**). This group includes the species *Staphylococcus* spp., a bacterium present in the skin microbiota, causing opportunistic infections, with great clinical relevance and known for its methicillin resistance (LAKHUNDI S, ZHANG K, 2018). Like Gram-positive Bacilli, Cocci has a significant presence in environments such as soil, where the acquisition and dissemination of resistance genes are favorable, confirming their prevalence in isolated strains due to lettuce preparation methods (SANTANA EHWD, et al., 2010).

The prominent presence of these three bacterial groups may indicate an important role of the environment (soil and water) in lettuce and other vegetable contamination. Microbiological contamination mainly occurs through contaminated water used for irrigating vegetables or even soil contamination caused by organic fertilizer, reinforcing the need for sanitary hygiene to preserve vegetable cultivation environments and other foods for consumer safety (CAUMO KS, et al., 2010).

It is evident that lettuce, like other vegetables, tends to be a potential vector for bacterial infections due to the presence of potential pathogenic strains, especially those resistant to the dilutions of hypochlorite recommended for lettuce hygiene, as observed in this study (BELTRAME CA, 2012).

In this context, there was variance in the CIM levels of the isolates obtained, with an average of approximately 0.1% (**Figure 1**), i.e., four times higher than the minimum inhibitory concentration for vegetables, and close to the concentration used for surface sanitation (ANVISA, 2008). The observed minimum concentration was 0.025%/L, which is the standard recommended by ANVISA for effective disinfection of vegetables, and the maximum concentrations were at 0.2%/L. According to ANVISA (2008), the concentration of 0.2%/L is recommended only for decontamination of semi-critical hospital articles, instruments that encounter non-intact skin and mucous membranes. Thus, the isolation of two strains with a minimum inhibitory concentration of 0.2%/L (data not shown) raises an alert for monitoring the efficiency of hygiene procedures, both for food and for disinfection in healthcare facilities, since hypochlorite is one of the main biocides used in sanitizing these locations (GILDO MGP, et al., 2017).

Another important finding regarding CIM was the high average resistance of Gram-positive Cocci, a group of great clinical relevance, with bacteria known to be opportunistic and multidrug-resistant, such as *Staphylococcus* spp., one of the major causes of food poisoning (CARMO LSD, et al., 2003). It is known that currently there are strains that escape the bactericidal spectrum of the currently used sanitizing compounds. Thus, these bacteria tend to exhibit multidrug resistance or co-resistance, such as *Staphylococcus* spp., and especially Enterobacteriaceae. Therefore, the performed antibiogram was essential to trace the phenotypic profile of possible multidrug resistances.(BRITO CBSD, et al., 2020)

The antibiogram tested susceptibility to first-line antibiotics such as amoxicillin, azithromycin, tetracycline, and ciprofloxacin. These drugs are commonly prescribed for various bacterial infections but are currently facing the challenge of reduced bacterial susceptibility, diminishing their effectiveness in certain therapies, and potentially becoming ineffective in more critical cases (COSTA ALP e JUNIOR ACSS, 2017).

In this context, the sensitivity profile (**Table 3**) showed approximately 40% of Enterobacteriaceae isolates susceptible to the tested antibiotics, while 6 identified phenotypes were resistant to at least one antibiotic, with 4 showing multidrug resistance between amoxicillin and another tested antibiotic. Resistance to amoxicillin is prevalent today, as evidenced by the resistance profile of bacteria isolated from urinary tract infections in hospitals in the North and Northeast regions, indicating the low efficacy of amoxicillin in treating patients. These findings demonstrate the loss of effectiveness of this drug against all other available therapeutic antibiotics (FURLAN APF, et al., 2021).

The identification of phenotypes with single resistance to azithromycin or tetracycline also highlights the diversity of resistant strains resulting from selective pressure from the irrational use of antibiotics and efficient bacterial genetic propagation (SILVEIRA MC, 2018). These two factors also favor the emergence of multidrug-resistant strains (Phenotypes D, E, F). Multidrug resistance is an alarming process, as the development of new antimicrobial compounds does not follow the same pace as bacterial adaptability[31], and it is estimated that by 2050, deaths from resistant bacteria will surpass those from cancer, reaching the mark of 10 million per year (IRIARTE DF, 2020).

Among the antibiotics tested, Ciprofloxacin proved effective against all strains, with no phenotype showing resistance to it. This fact can be explained by the efficiency demonstrated by this antibiotic against gram-negative bacteria, as well as its limited use in the community setting, being more restricted to conscientious medical prescription (SOUZA RB, et al., 2010).

In this context, it was investigated whether resistance to each tested antibiotic was influenced by tolerance to hypochlorite, as the process of co-resistance between biocides and antibiotics has been described (ELEKHNAWY E, et al., 2020). For this purpose, a Pearson correlation test was conducted between CIM values for Sodium Hypochlorite and the diameters of the halos observed in the disc diffusion test for each antibiotic. Consequently, an inverse and possible correlation was observed between the halos of amoxicillin and the levels of hypochlorite resistance, meaning that the higher the CIM, the smaller the halo formed by the strain in the diffusion disc.

This correlation may be related to two factors: 1) The widespread dissemination of beta-lactam genes in the environment due to the indiscriminate use of penicillin derivatives; 2) The wide range of resistance mechanisms that increase tolerance to hypochlorite and consequently to other antimicrobials, which are part of the process of cross-resistance (FURLAN APF, et al., 2021)..

Cross-resistance is the result of physiological adaptations that have effects on various antimicrobial compounds. An example of such adaptations is the overexpression or upregulation of efflux pumps and reduced permeability of the cell membrane, resulting in more generalist resistance mechanisms (ELEKHNAWY E, et al., 2020). No studies indicating cross-resistance between sodium hypochlorite and amoxicillin were observed. However, several studies highlight the relationship between tolerance to Benzalkonium Chloride (a chlorinated compound) and resistance to amoxicillin (FERREIRA MCS, 2019).

Additionally, it is noteworthy that approximately 50% of Enterobacteriaceae strains (all tolerant to hypochlorite) show resistance to at least one antibiotic. The misuse of biocidal agents may enrich antibiotic resistance among gram-negative bacterial species, emphasizing that these biocides should be restricted to applications with proven health benefits (FERREIRA MCS, 2019).

CONCLUSION

The use of sodium hypochlorite at a concentration of 0.025% demonstrated significant efficacy in reducing mesophilic bacteria in vegetables, highlighting its relevance for food sanitization. However, the identification of resistant bacterial strains, particularly among Gram-positive Bacilli and Enterobacteriaceae groups, underscores the need for greater surveillance regarding bacterial adaptation to biocides. Additionally, when correlating hypochlorite resistance with antibiotic resistance, the size of amoxicillin inhibition zones showed an inverse correlation with MIC levels, suggesting a potential mechanism of cross-resistance. This study demonstrated that sanitization with sodium hypochlorite is necessary to reduce Foodborne Diseases (FBDs) caused by bacteria. Nevertheless, the identification of strains resistant to the MIC recommended for vegetable sanitization, especially those exhibiting multidrug-resistant antibiotic phenotypes, raises concerns about bacterial adaptation and the potential for increased severity and incidence of FBDs.

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