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Phenotypic and genotypic resistance of *Staphylococcus aureus* in fresh meats marketed in Pernambuco, Brazil

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Abstract

Antimicrobial resistance is a challenge for public health, exacerbated by the indiscriminate use of these agents in humans and animals. *Staphylococcus aureus*, frequently found in fresh meat, poses a considerable risk due to its ability to transfer resistance genes. This study assessed the phenotypic and genotypic resistance profiles of *Staphylococcus aureus* isolated from 120 samples of beef, pork, and chicken sold in Recife, Pernambuco, Brazil. Samples were collected from markets, butcher shops, and street markets. Phenotypic identification was conducted using catalase tests and Gram staining, with confirmation through polymerase chain reaction (PCR) by amplifying the *nuc* gene. The resistance profile was evaluated using the disk diffusion method for 21 antimicrobials, following Clinical and Laboratory Standards Institute guidelines. In contrast, the resistance genes *blaZ*, *mecA*, *mecC*, *norA*, *norC*, *tet(38)*, and *msrA* were analyzed by PCR. Among the 152 *Staphylococcus* spp. colonies isolated, 6.67% (10/152) were confirmed as *S. aureus*. Of these, 70% (7/10) were isolated from pork and 30% (3/10) from chicken, with no detection in beef samples. All isolates exhibited multidrug resistance, showing universal resistance to penicillin, rifampin, tetracycline, doxycycline, erythromycin, clindamycin, and linezolid. Genotypically, all isolates carried the *norC* gene, while 90% (9/10) harbored *tet(38)*, and 60% (6/10) tested positive for *norA* and *blaZ*. No detection of *mecA*, *mecC*, or *msrA* was observed. These results underscore the urgent need for health education promoting the rational use of antimicrobials and coordinated actions between human and animal health sectors to mitigate antimicrobial resistance, which poses a serious threat to public health.

Keywords: antimicrobial resistance; multidrug-resistant bacteria; food safety; animal production.

Practical Application: Human health risks associated with the consumption of meat contaminated with antimicrobial-resistant *S. aureus*.

1 INTRODUCTION

Bacterial resistance to antimicrobials is widely recognized as one of the most significant challenges to global public health (Mota et al., 2005; World Health Organization [WHO], 2022). Research by Antimicrobial Resistance Collaborators (2022) estimates that approximately 700,000 annual deaths are caused by infections with resistant bacteria, with alarming projections suggesting this number could rise to 10 million deaths annually by 2050 if effective measures to control and prevent the emergence of multidrug-resistant strains are not implemented (Antimicrobial Resistance Collaborators, 2022; Brasil, 2021a).

The emergence of multidrug-resistant bacterial strains has been linked to the indiscriminate use of antimicrobials, both in animal production and through human self-medication (Costa et al., 2013). Bacterial resistance mechanisms, such as enzyme production and efflux systems, may be intrinsic or acquired. These mechanisms work by inactivating antimicrobials, interfering with their functions, or utilizing transport proteins in the bacterial cell membrane to expel substances from different classes, thereby protecting bacteria from their lethal effects (Moreira et al., 2008).

Microorganisms of the *Staphylococcus* genus are commonly found in the respiratory, urogenital, and digestive tracts of humans and animals, where they can act as opportunistic pathogens (Crespo-Piazuelo & Lawlor, 2021). This genus is associated with a wide range of waterborne and foodborne diseases, as well as zoonoses and anthropozoonoses (Lee et al., 2021).

Fresh meat, due to its high-water activity and nutrient content, provides a favorable environment for microbial proliferation when contaminated, particularly for toxin-producing microorganisms, which can pose severe risks to public health (Húngaro et al., 2016). Meat products are highly susceptible to

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bacterial contamination at all stages of the production chain—from slaughter to transportation, storage, and consumption. Contamination may involve various pathogenic and non-pathogenic microorganisms (Luz et al., 2017), with *S. aureus* being of particular concern due to its capacity to cause foodborne illnesses (FBI) and transfer antimicrobial resistance genes (Carvalho et al., 2012).

Given the public health implications of multidrug-resistant *S. aureus*, it is essential to understand the epidemiological and pathogenic profile of this bacterium, given its importance for human, animal, and environmental health. Therefore, this study aimed to investigate the phenotypic and genotypic profiles of *S. aureus* strains isolated from chicken, beef, and pork meat sold for consumption in the city of Recife, Pernambuco, Brazil.

1.1 Relevance of the work

The presence of resistant *Staphylococcus aureus* in meat sold in Recife, Brazil, underscores a risk, given that this bacterium functions as a foodborne pathogen and a reservoir for resistance genes. Monitoring antimicrobial-resistant *S. aureus* is essential for tracking resistance patterns within the production chain and implementing interventions that protect consumer health.

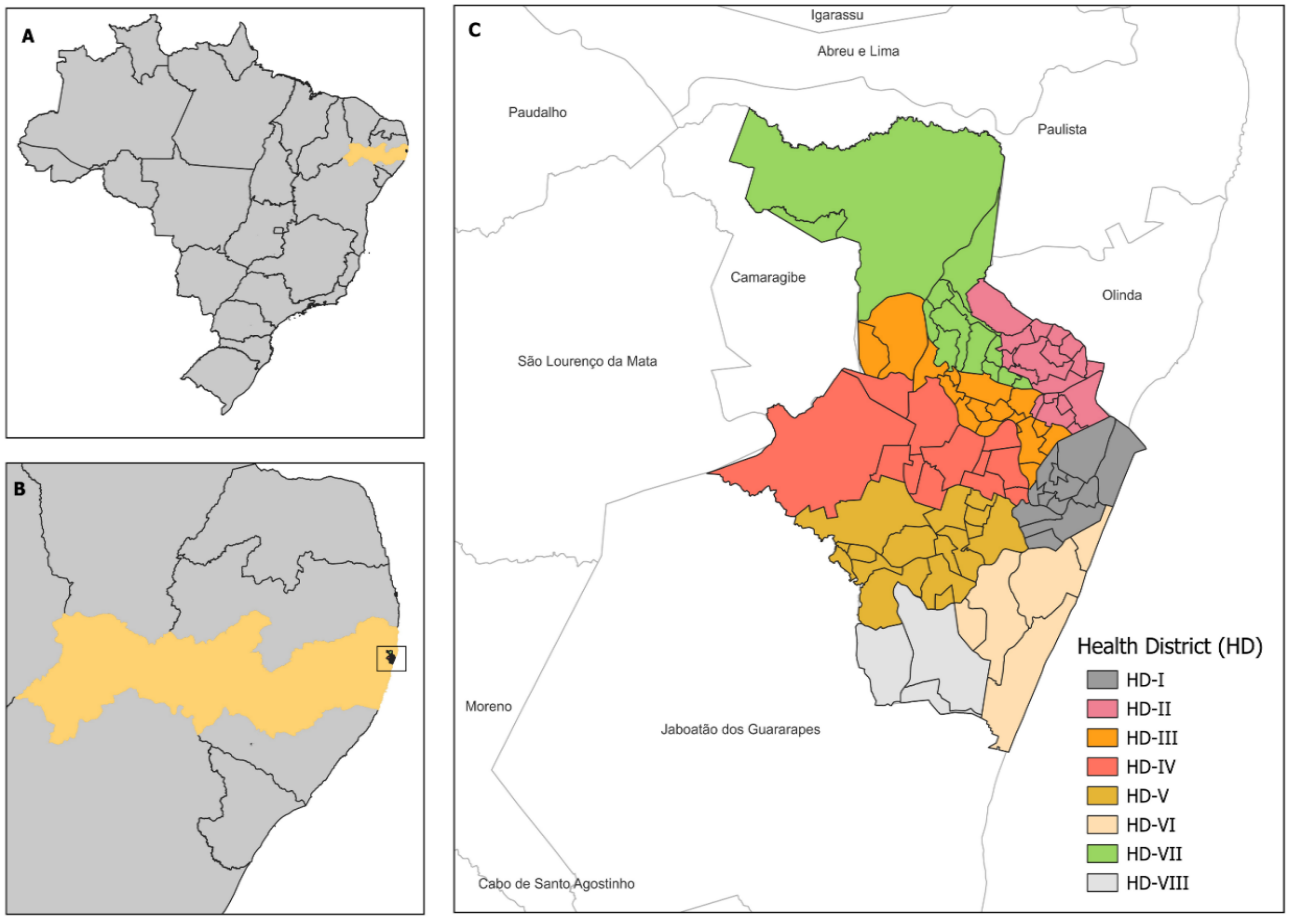
2 MATERIALS AND METHODS

2.1 Sample collection

A total of 120 fresh meat samples were collected, comprising 40 beef, 40 pork, and 40 chicken samples. All samples were obtained from commercial establishments, including markets, butcher shops, and street markets, located within the eight Health Districts of Recife, Pernambuco (Figure 1). Sampling was performed using a non-probabilistic convenience sampling method (Vehovar et al., 2016). The samples were labeled and transported to the laboratory in isothermal containers with reusable ice packs, ensuring a temperature range of 2°C to 8°C during transit. Upon arrival, the samples were subjected to microbiological and molecular processing.

2.2 Identification of Staphylococcus aureus

Initially, 25 ± 0.2 g of each sample was weighed and transferred to sterile Stomacher bags containing 225 mL of 0.1% peptone water. The samples were homogenized for 60 s and incubated at 37°C in a microbiological incubator under aerobic conditions for 18 to 24 h. Following incubation, the samples were plated onto mannitol salt agar and incubated again at 37°C for 24 h in a bacteriological incubator (Koneman et al., 2018).



(A) Brazil and the State of Pernambuco; (B) the State of Pernambuco and the city of Recife; (C) the Health District of the city of Recife.

Figure 1. Health Districts of Recife, Pernambuco.

To confirm the bacterial species, three to five morphologically compatible colonies were selected and streaked onto fresh mannitol salt agar plates, followed by incubation at 37°C for 24 to 48 h. Phenotypic identification of colonies was performed based on the presence of typical rounded, grayish or yellowish colonies with mannitol fermentation, indicative of *S. aureus* (Džidić et al., 2008).

Subsequently, catalase testing and Gram staining were performed to observe the morphotintorial characteristics of the bacterial genus (Koneman et al., 2018). Confirmation of *S. aureus* species was carried out through DNA extraction and amplification of the specific *nuc* gene region by PCR (González-Domínguez, et al. 2020).

2.3 Phenotypic profile of antimicrobial resistance

The *S. aureus* isolates were subjected to phenotypic antimicrobial susceptibility testing using the disk diffusion method, following the guidelines provided in the Clinical and Laboratory Standards Institute (CLSI) manual (CLSI, 2020).

The tests were conducted on Mueller-Hinton agar using the following antimicrobials: penicillin (10 IU), penicillin + novobiocin (40 µg), ampicillin (10 µg), imipenem (10 µg), amoxicillin + clavulanic acid (20+10 µg), sulfamethoxazole + trimethoprim (25+5 µg), rifampin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), enrofloxacin (5 µg), chloramphenicol (30 µg), doxycycline (30 µg), gentamicin (10 µg), linezolid (10 µg), neomycin (30 µg), erythromycin (15 µg), clindamycin (2 µg), cefoxitin (30 µg), tetracycline (30 µg), and ceftiofur (30 µg).

Before inoculating colonies onto Mueller-Hinton agar, all isolates were suspended in saline solution, with the turbidity adjusted to match a 0.5 McFarland standard. After applying the antimicrobial discs to the agar plates, the samples were incubated at 37°C for 18 to 24 h. Zone diameters of inhibition were measured, and the results were interpreted following the CLSI VET01S manual (CLSI, 2020).

2.4 Analysis of genetic determinants of antimicrobial resistance

The *S. aureus* isolates were streaked onto mannitol salt agar to obtain pure colonies in sufficient quantities for DNA extraction. DNA extraction was performed using the thermal lysis method as described by Kyselková et al. (2015). The extracted DNA was quantified, and its purity was assessed using a spectrophotometer (Thermo Scientific Multiskan Go) with absorbance readings at 260 nm. The DNA concentration was adjusted to 100 ng/µL for subsequent analyses.

To detect resistance genes in the *S. aureus* isolates, PCR was employed to amplify the *blaZ* gene, aiming to identify strains with the potential to produce beta-lactamase, an enzyme capable of hydrolyzing the beta-lactam ring (Sawant et al., 2009). Additionally, the *mecA* and *mecC* genes, which confer resistance to methicillin, were investigated to characterize the isolates as methicillin-resistant *S. aureus* (MRSA) (Nakagawa et al., 2005; Paterson et al., 2012).

The genes *tet(38)*, *norA*, *norC*, and *msrA* were also screened to assess the genetic potential for encoding multidrug efflux systems (Floyd et al., 2010; Martineau et al., 2000; Truong-Bolduc et al. 2003, 2005, 2006). The specific primers used for amplifying these resistance-related genes are listed in Table 1.

3 RESULTS

Of the bacterial isolates obtained from beef, pork, and chicken samples, 10 out of 152 (6.67%) were confirmed as *S. aureus* through PCR targeting the *nuc* gene. Among these, 7/10 (70%) were isolated from pork and 3/10 (30%) from chicken, with no *S. aureus* detected in beef samples. Of the positive isolates, 6/10 (60%) originated from Health District VI, while Health Districts II, V, VII, and VIII each accounted for 1/10 (10%) of the isolates.

The results of the phenotypic resistance tests for the 10 *S. aureus* isolates are presented in Figure 2. All isolates were classified as multidrug-resistant, as they exhibited resistance to more than three different antimicrobial classes, including β-lactams, rifamycins, tetracyclines, aminoglycosides, macrolides, and lincosamides.

A prevalence of 60% (6/10) of MRSA was identified among the analyzed isolates. These MRSA strains were obtained from Health Districts V (2 isolates), VI (3 isolates), and VIII (1 isolate), originating from four pork samples and two chicken samples.

Table 1. Primers used to identify genes encoding antimicrobial resistance.

Target gene	Primer	Sequence (5′- 3′)	Amplicon (PB)	References
blaZ	blaZ – F blaZ – R	AAGAGATTTGCCTATGCTTC GCTTGACCACTTTTATCAGC	517	Sawant et al. (2009)
*mec*A	2W 2X	TGGTATGTGGAAGTTAGATTGGGAT CTAATCTCATATGTGTTCCTGTATTGGC	155	Nakagawa et al. (2005)
mecC	1[a] 1B	CATTAAAATCAGAGCGAGGC TGGCTGAACCCATTTTTGAT	188	Paterson et al. (2017)
*nor*A	norA – F norA – R	TGCAATTTCATATGATCAATCCC AGATTGCAATTCATGCTAAATATT	150	Truong-Bolduc et al. (2003)
*nor*C	norC – F norC – R	ATAAATACCTGAAGCAACGCCAAC AAATGGTTCTAAGCGACCAA	200	Truong-Bolduc et al. (2006)
*tet*38	tet-38 – F tet-38 – R	TTCAGTTTGGTTATAGACAA CGTAGAAATAAATCCACCTG	200	Truong-Bolduc et al. (2005)
*msr*A	msrA – F msrA – R	TCCAATCATTGCACAAAATC AATTCCCTCTATTTGGTGGT	890	Martineau et al. (2000)

Genotypic analysis of the antimicrobial resistance profiles of *S. aureus* isolates demonstrated that 100% (10/10) of the isolates carried the *norC* gene, while 90% (9/10) harbored the *tet(38)* gene. Additionally, 60% (6/10) of the isolates tested positive for both the *norA* and *blaZ* genes. No detection of the *mecA*, *mecC*, or *msrA* genes was observed in any of the isolates (Figure 3).

4 DISCUSSION

This study revealed that *S. aureus* was confirmed in 6.67% of the bacterial isolates obtained from fresh meat, predominantly in pork (70%) and chicken (30%), with no detection in beef samples. All *S. aureus* isolates demonstrated multidrug resistance, showing resistance to more than three classes of antimicrobials, including β-lactams, rifamycins, and tetracyclines. Genotypic analysis identified the resistance genes *norC*, *tet(38)*, *blaZ*, and *norA*, indicating variability in both phenotypic and genotypic resistance profiles. These findings underscore the presence of multidrug-resistant *S. aureus* in foods of animal origin and highlight the importance of monitoring antimicrobial resistance in food production systems. This study provides the first dataset on antimicrobial resistance in fresh meat from a region where such information is limited, reinforcing the need for surveillance and control measures.

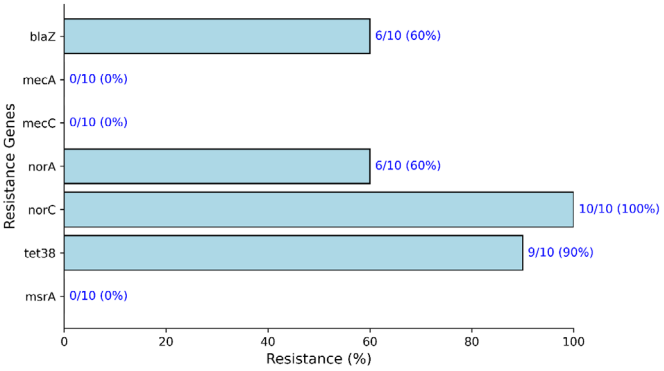


Figure 3. Genetic determinants of antimicrobial resistance in *Staphylococcus aureus* isolates from commercial pork and chicken meat samples.

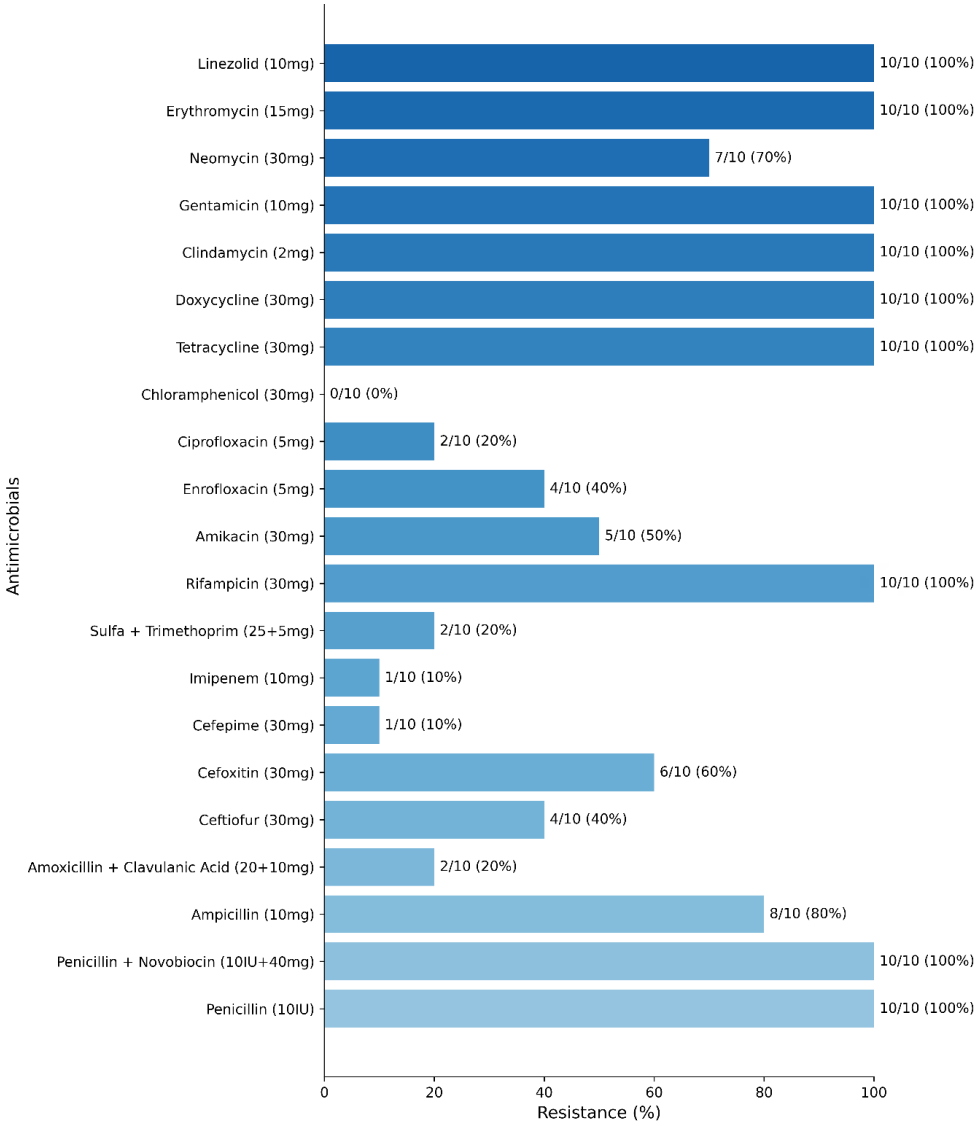


Figure 2. Results of phenotypic antimicrobial resistance analysis of *Staphylococcus aureus* isolates from commercial pork and chicken meat samples.

The predominance of *S. aureus* in pork and chicken samples suggests these meats may pose a greater risk than beef. This finding contrasts with studies in the literature reporting higher contamination rates across all meat types. For instance, Abdalrahman et al. (2015) and Wu et al. (2018) observed *S. aureus* prevalence rates of up to 67.9% in raw poultry and 60.9% in frozen poultry. In beef, contamination rates ranged from 50.4% to 80% in specific cuts, such as liver (Abdalrahman et al., 2015; Kadariya et al., 2014). Raw pork showed prevalence rates of up to 47.7%, while frozen pork reached 50% (Wu et al., 2018).

The discrepancy between our findings and the literature may stem from regional differences in meat processing and storage practices, as well as variations in study methodologies. Poor handling practices, such as insufficient cooking or prolonged exposure to unsafe temperatures, increase contamination risks (Kadariya et al., 2014; Rajaei et al., 2021). Additionally, *S. aureus* is capable of surviving on kitchen surfaces and utensils, facilitating cross-contamination during food preparation (Howden et al., 2023; Kadariya et al., 2014).

Similar to our findings, Cerqueira and Almeida (2013) confirmed the presence of multidrug-resistant *S. aureus* in raw meat in a study conducted in Minas Gerais. Studies by Silva et al. (2021) and Aragão et al. (2021) in Pernambuco further demonstrated the isolation of multidrug-resistant *S. aureus* in milk, cheese, and goat's milk, emphasizing the role of farm animals as reservoirs of resistant bacteria. The emergence of resistant *S. aureus* isolates is often attributed to the misuse of antimicrobials in animal production, posing significant threats to both human and animal health (Lima et al., 2017).

The spread of resistant bacteria among food handlers in environments such as slaughterhouses, dairies, and industrial kitchens poses a public health risk. Workers in contact with meat, milk, or dairy products are frequently exposed to zoonotic pathogens, including *S. aureus*, increasing the potential for workplace transmission of resistant strains, such as MRSA. This can result in cross-contamination and transmission to end consumers (Ferreira et al., 2014; Silva et al., 2020; Soares et al., 2012; Strommenger et al., 2018). High contamination rates among food handlers have been documented in Brazil. For instance, Soares et al. (2012) found *S. aureus* on the hands of 53.3% (88/166) of food handlers in public schools in Camaçari, Bahia, due to inadequate hygiene practices. Similarly, Ferreira et al. (2014) reported that 50% (70/140) of samples from food handlers' hands and nostrils in Salvador hospitals were positive for *S. aureus*, with 28.6% (20/70) being MRSA.

In this study, 60% (6/10) of the *S. aureus* isolates were identified as MRSA. Similarly, Costa et al. (2015) found MRSA in 28.1% (32/114) of raw meat samples and 9.5% (6/63) of cooked meat samples analyzed in Salvador hospitals, demonstrating that this pathogen can survive under various processing conditions. Other Brazilian studies report consistent MRSA incidences in foods of animal origin, particularly in meat and dairy products, indicating a national problem (Alves et al., 2018; Dittmann et al., 2017; Monte et al., 2018).

The presence of antimicrobial-resistant *S. aureus* in the food supply chain represents a significant threat to public health, both in Brazil and globally. This bacterium is a leading cause of FBI in Brazil, as reported by the Health Surveillance Secretariat (Brasil, 2021c). Infections can range from mild gastrointestinal discomfort to severe systemic conditions. Research indicates that *S. aureus* can exert cytotoxic effects on human intestinal cells, reducing cell viability and mitochondrial membrane potential, which underscores the potential severity of such infections (Merghni et al., 2023). Multidrug resistance further complicates treatment, limiting therapeutic options (Jia et al., 2020).

Global health organizations, including the Agência Nacional de Vigilância Sanitária ([ANVISA], Brasil, 2021b; 2022), and WHO (2022) emphasize the catastrophic potential of antimicrobial resistance and project a sharp rise in related deaths by 2050 if control measures are not implemented (Antimicrobial Resistance Collaborators).

The absence of *mecA* and *mecC* genes in cefoxitin-resistant *S. aureus* isolates suggests the involvement of alternative resistance mechanisms. One possibility is β-lactamase hyperproduction, which hydrolyzes β-lactam antibiotics, rendering them ineffective (Argudín et al., 2018). Mutations in penicillin-binding protein genes, particularly *pbp4*, can also reduce affinity for β-lactams, contributing to resistance. Nonsense mutations in *pbp* genes have been documented in resistant strains (Mlynarczyk-Bonikowska et al., 2022). Alterations in cell wall metabolism and auxiliary gene expression (*femX*, *femA*, and *femB*) further enhance resistance (Liang et al., 2022). Other regulatory mechanisms, such as random genetic mutations and recombination events, may also play a role but were not assessed in this study (García-Álvarez et al., 2011; Moreira et al., 2018; Virdis et al., 2010).

The detection of the *blaZ* gene in *S. aureus* isolates from pork and chicken highlights the risk of β-lactam resistance due to enzyme production. If transmitted to humans, infections caused by these strains may require prolonged hospitalizations and could result in death in severe cases (Alghamdi et al., 2023; Frosini et al., 2020; Shore et al., 2011). Contamination with *blaZ*-carrying bacteria likely occurs during processing and marketing or through prolonged confinement of animals, which facilitates the spread of resistant strains and environmental contamination (Freitas et al., 2001; Kahn, 2016).

Efflux pump genes detected in this study (*norC*, *tet(38)*, *norA*, and *msrA*) have also been reported in northeastern Brazil in studies of dairy cattle and goat herds (Aragão et al., 2021; Silva et al., 2021). This reinforces the need to explore the association between these genes and difficult-to-treat infections in humans and animals.

The contamination and dissemination of multidrug-resistant bacteria may be attributed to close contact between production animals and their handlers, as suggested by Silva et al. (2022). Fagundes et al. (2010) highlight that cross-contamination between animals, humans, and food can occur due to inadequate hygiene of utensils used in animal handling. Resistant bacteria can spread from carrier animals to non-carriers, during trade or transport, and between humans and animals, posing risks to public health.

The results of this study confirm the occurrence of multidrug-resistant *Staphylococcus aureus* in pork and chicken meat sold in Recife, highlighting the critical need for public health strategies that promote the prudent use of antimicrobials in livestock production. In addition, the results reinforce the need to implement strict biosafety and hygiene protocols throughout the food production process, from slaughter to points of sale, to contain the spread of antimicrobial-resistant bacteria, which represent a growing concern for health and financial systems.

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