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Hygiene conditions of mussels *Perna perna* captured in Niterói, RJ, Brazil: thermal intervention and microbiological evaluation

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Abstract

Bivalve molluscs are important fishing resources for human consumption around the world. However, for safe consumption, good hygiene conditions must be ensured throughout the production chain. Eating bivalve molluscs, particularly raw, can pose a significant health risk to consumers because they can act as vectors of infectious pathogens. This study aimed to evaluate the microbiological quality of mussels from Niterói, RJ and the use of cooking to ensure food safety, as well as assessing the susceptibility of *Escherichia coli* and coagulase-positive *Staphylococcus* strains to different antimicrobials. Therefore, microbiological analyzes were performed on mussels *Perna perna* before and after thermal processing, the impact of cooking on the microorganisms was verified. Cooking the mussels in water at 95°C for 1 (one) minute reduced product contamination. The use of the time x temperature binomial was sufficient to reduce pathogenic microorganisms to acceptable levels according to Brazilian legislation, although the pathogens were only completely eliminated after five minutes. The bacteria investigated had a high antibiotic resistance index: 66% of *E. coli* strains and 34.78% of coagulase-positive *Staphylococcus* strains were found to be resistant to multiple drugs.

Keywords: bivalve molluscs; thermal processing; cooking; pathogenic microorganisms; antimicrobials.

Practical Application: Mussels are filtering organisms, widely used as a bioindicator. On the other hand, they are highly appreciated delicacies in cooking. Thus, it is important that the population feels safe when consuming it. The present study seeks to guide the population about the microbiological hazards and contributes to other studies so that the minimum cooking time required for mussels can be included in the labeling of this product.

1 Introduction

Bivalve molluscs are important sources of food and income for fishing communities, playing an important socioeconomic and cultural role (Legat et al., 2010). They are considered a prominent commodity of high nutritional value and are widely used for human consumption (Daltro et al., 2013).

These organisms are responsible for several epidemic outbreaks (Daltro et al., 2013), as they may bioaccumulate in their tissues several microorganisms present in the water (Brasil, 2014; Ballesteros et al., 2018). However, consumers' perception of food risks is multifactorial, ranging from social, cultural, psychological, ethical to moral aspects. In addition to technical-scientific knowledge, subjective issues contribute to the perception of risks, considering the benefits that some food provides (Rembischevski & Caldas, 2020).

Thus, the lack of basic sanitation, as well as poor hygiene during food processing can represent a risk to the health of consumers (Mafra et al., 2016). Moreover, genetic alterations in pathogenic microorganisms in fish are vectors for the emergence of multi-drug resistant microorganisms and a possible consequence of the spreading of their waste around ocean currents (Cordeiro et al., 2020).

High antibiotic resistance indexes in foodborne bacteria pose a significant health risk to consumers, as lower susceptibility to these drugs implies that there are fewer therapies available to treat possible infections caused by eating contaminated food (Cordeiro et al., 2020).

Therefore, the consumption of undercooked bivalve molluscs can cause diseases, especially when processed in poor hygiene conditions or when they come from contaminated environments (Barros et al., 2005). Thermal treatment at 100 °C for approximately 15 minutes is effective to reduce microbial load on mussels (Pereira et al., 2007).

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This study aimed to evaluate the microbiological quality of mussels from the city of Niterói, RJ and the use of cooking to ensure food safety, as well as assessing the susceptibility of *Escherichia coli* and coagulase-positive *Staphylococcus* strains to different antimicrobials.

2 Material and methods

Mussels (*Perna perna*) were collected in Baía da Guanabara (22° 52'13" S 43°9'53" W) by a community from Niterói, Rio de Janeiro. Nine (9) collections were carried out, with 1 kg of precooked packaged mussels and 60 animals (males and females) that were still alive collected (American Public Health Association, 2001).

The mussels were refrigerated in clean isothermal boxes with recyclable ice and taken to the Laboratory for Microbiological Control of Animal Origin Products (CMPOA), of Universidade Federal Fluminense. The still alive mussels were washed under running water, with a brush, air dried after the process and sent for microbiological analysis (American Public Health Association, 2001).

In addition to the group of fresh animals, the mussels were divided into five groups of animals weighing approximately 200 g: control (precooked by shellfish collectors) and four thermal treatments. The groups that underwent thermal processing in the laboratory were placed in water heated to a temperature of 95 °C ± 1 °C, measured with a food thermometer (XT-1234, XTRAD®). They were divided into four groups and cooked for different times: 1 min (T1), 3 min (T3), 5 min (T5) and 7 min (T7).

The temperature of 95 °C aimed to simulate cooking conditions in homes where the formation of small bubbles indicate the boiling point of water was reached and the mussels can be added to the pot.

Mussel analyzes included count of Aerobic Mesophilic Heterotrophic Bacteria (CAMHB), search for *Salmonella* spp. and count of coagulase-positive *Staphylococci,* according to the APHA methodology described in the "Compendium of Methods for the Microbiological Examinations of Foods" (Salfinger & Tortorello, 2015).

For the analysis of coliforms and *E. coli*, a fast miniaturized technique in multiple tubes, according to Merck Microbiology (2000), modified by Franco & Mantilla (2004) was used, and Fluorocult broth was the culture medium (Himedia®, code M1453).

The results were compared with the provisions of IN 60 (Normative Instruction. 60, of December 23, 2019, of ANVISA), based on the classification of fish species, since although mussels are bivalve molluscs, they are not consumed live (raw) (Brasil, 2019).

E. coli and coagulase-positive *Staphylococcus* strains were tested for susceptibility to antimicrobials, according to the recommendations of Clinical and Laboratory Standards Institute (2021). The bacterial suspension was standardized according to the Kirby-Bauer method (Bauer et al., 1966), with turbidity adjusted with sterile saline solution, corresponding to 1.0 Mc Farland standard, in a total of 3x108 bact/mL of medium.

The inoculum was seeded on plates containing Müeller Hinton Agar with the aid of a sterilized swab. With previously flamed and cooled forceps, Sensifar vet sensitivity discs (Cefar® brand) were applied on the culture medium containing the inoculum. The following antimicrobials were tested: tetracycline (TET - 30 mcg), gentamicin (GEN - 10 mcg), chloramphenicol (CLO - 30 mcg), ciprofloxacin (CIP - 5 mcg), norfloxacin (NOR - 10 mcg) and nitrofurantoin (NIT - 300 mcg) for both bacteria.

For *E. coli*, ampicillin (AMP - 10 mcg), cefuroxime (CRX - 30 mcg), cephalexin (CFE - 30 mcg) and cephalothin (CFL - 30 mcg) were also tested. Also, clindamycin (CLI - 2 mcg) and erythromycin (ERI - 15 mcg) were chosen exclusively for coagulase-positive *Staphylococcus*.

The plates were incubated at 35-37 °C for 18-24 hours and inhibition halos were observed, which were measured with a halometer (millimeters). Standard measures of the inhibition zones of each antimicrobial were used in the interpretation of the test, according to a table established by Clinical and Laboratory Standards Institute (2021) that classifies each strain as resistant, intermediate or susceptible.

Exploratory analysis of the variables was performed in statistical analysis, and Friedman and Wilcoxon nonparametric tests were used, both for paired samples. The global significance level adopted was 5% and, for multiple comparisons (comparisons of 2 groups) between the time intervals assessed, a significance level of 0.4% was used in each assessment, according to Bonferroni's correction proposal.

3 Results and discussion

The results of the analyzes of fresh and processed mussels submitted to different cooking times are described in Table 1.

CAMHB ranged from 1.2 x 10^3 to 7.4 x 10^4 CFU/g in fresh mussels and from 7.7 x 10^3 to 5.1 x 10^6 CFU/g in precooked mussels (control). A significant difference ($p < 0.001$) was found between the intervals of time evaluated regarding CAMHB. The most significant differences were found between the fresh or precooked product (control) and the products observed after three or more minutes of cooking. This is consistent with Nascimento et al. (2011), who reported values ranging from 10^3 to 10^7 CFU/g for the fresh product. The authors found similar values, of 10^6 CFU/g in the precooked product.

Increase in CAMHB after processing was expected, as according to Daltro et al. (2013), appropriate hygiene measures are not observed by the people who extract product, and handling after capture directly influences the microbiological quality of the end product.

After cooking by immersion in water at 95 °C for one minute, CAMHB ranged from 1.0 x 10^2 to 8.9 x 10^4 CFU/g, showing that proper thermal treatment can reduce microbial load on these products. The count ranged from 7.1 x 10 to 1.7 x 10^3 CFU/g after three minutes. At five minutes, it remained between 1 x 10 to 1.3 x 10^3 CFU/g and between 1x 10 to 1.2 x 10^3 CFU/g after seven minutes. After three minutes of cooking, the mean was practically constant, in 10^2 CFU/g. Despite a slight

reduction in CAMHB, extra cooking time is not necessary at a temperature of 95 °C.

Lower counts were obtained by Nascimento et al. (2011) who submitted Charru mussels and oysters to steam precooking or immersion in water at 95 °C for 05, 10 and 15 minutes after opening the shells and found CAMHB values < 10 CFU/g. However, in this case, the bivalves were in the shells and the precooking process observed good hygiene practices, unlike the present study where the mussels sampled had been previously precooked by the shellfish collectors.

Analysis of the samples based on the referred IN 60 (Brasil, 2019) regarding coagulase-positive *Staphylococci* count for fresh and precooked mussels (control) is described in Figure 1.

In fresh animals, the coagulase-positive *Staphylococci* count ranged from 1.7 x 10^2 CFU/g to 3.4 x 10^5 CFU/g. There was a significant difference ($p < 0.001$) between the intervals of time evaluated for this count. The most significant differences were observed between the fresh or precooked product (control) and the products observed after cooking. Similar results were reported by Silva et al. (2021) in Piúma (ES), who found a density of *S. aureus* ranging from 1.28 x 10^2 to 4.35 x 10^3 CFU/g in mussels *Perna perna*.

According to IN 60, a maximum value of 10^3 CFU/g in fish is recommended for this microorganism. The quality of a product with values ranging between 10^2 to 10^3 CFU/g is classified as intermediate (Brasil, 2019).

Therefore, 44.46% of fresh mussel samples were unacceptable and 55.6% had intermediate quality. The number of unacceptable samples increased, with 88.9% (8) in precooked (control) mussels and counts ranging from 5 x 10^2 CFU/g to 4.8 x 10^6 CFU/g. The levels of CFU/g obtained were higher than those described by Freitas et al. (2015), who reported that 33.33% of Charru mussels precooked by shellfish collectors was contaminated with *S. aureus* at levels above those permitted by law, and that the tools were also contaminated by *E. coli* in 11.11% of the samples.

According to Freitas et al. (2015), the main cause of contamination of processed bivalves is the lack of proper handwashing by shellfish collectors and improper cleaning of tools, which can cause contamination especially by coliforms and *S. aureus*. This is consistent with the present study where greater contamination of mussels was detected after their processing by fishermen, indicating that their hands and/or the tools used by the workers can be sources of recontamination of the product.

The judgment of mussels based on IN 60 (Brasil, 2019), regarding the count of coagulase-positive *Staphylococci* after thermal treatments in the laboratory is described in Figure 2.

After the one-minute thermal treatment, 77.8% (7) of the samples became acceptable, while 22.2% (2) showed intermediate quality, with a maximum count of 3 x 10^2 CFU/g. After three minutes of treatment, 88.9% (8) of the samples were acceptable, and 11.1% (1) had intermediate quality, with a maximum count of 1 x 10^2 CFU/g. However, despite the lower counts, it is still

Table 1. Mean bacteriological count values of mussels *Perna perna* in the different groups fresh, precooked (control), T1 (cooking 1 min), T3 (cooking 3 min), T5 (cooking 5 min) and T7 (cooking 7 min).

	FRESH	CONTROL	T1	T3	T5	T7	*p*
CAMHB (CFU/g)	$1.1 \times 10^4{}_a$	$1.1 \times 10^6{}_a$	$1.9 \times 10^4{}_{ab}$	$5.7 \times 10^2{}_b$	$3.8 \times 10^2{}_b$	$2.7 \times 10^2{}_b$	< 0.001
Coagulase- positive *Staphylococcus* (CFU/g)	$4.2 \times 10^4{}_a$	$1.1 \times 10^6{}_b$	$5.5 \times 10_c$	$1.1 \times 10_c$	$< 100_c$	$< 100_c$	< 0.001
Total coliforms (MPN/g)	$5.1 \times 10^3{}_a$	$2.6 \times 10^3{}_{ab}$	$4.4 \times 10_{ab}$	$< 3_b$	$< 3_b$	$< 3_b$	< 0.001
E. coli (MPN/g)	$2.5 \times 10^{2*}$	$2.3 \times 10_*$	$< 3_*$	$< 3_*$	$< 3_*$	$< 3_*$	< 0.001

CFU/g = Colony Forming Unit per gram. MPN/g = Most Probable Number per gram. *No significant difference was found in multiple comparisons. The letters identify the differences between the groups.

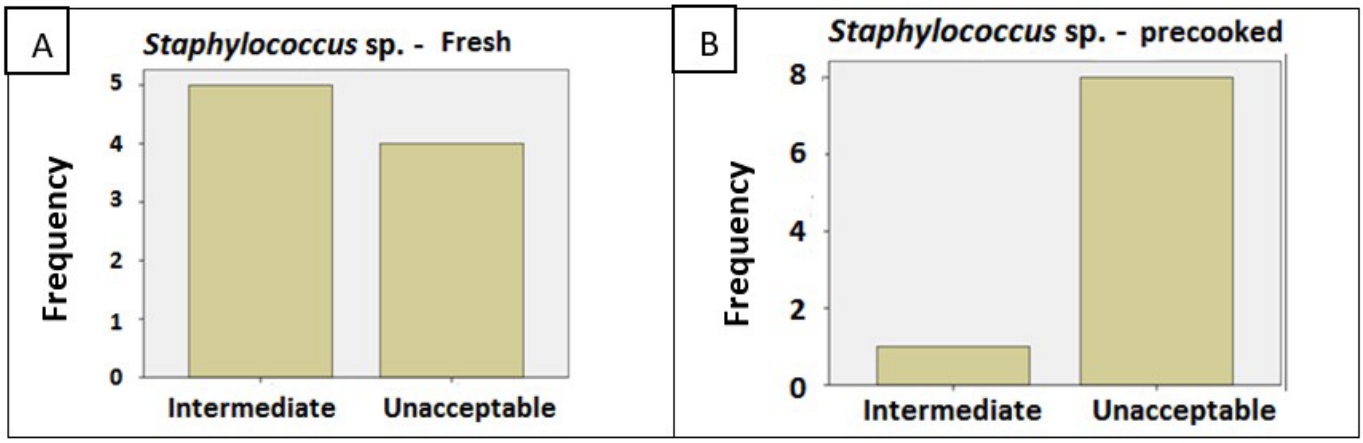

Figure 1. Judgment of mussel samples according to IN 60 (Brasil, 2019), based on the coagulase-positive *Staphylococci* count for fresh (A) and precooked (control) (B) mussels.

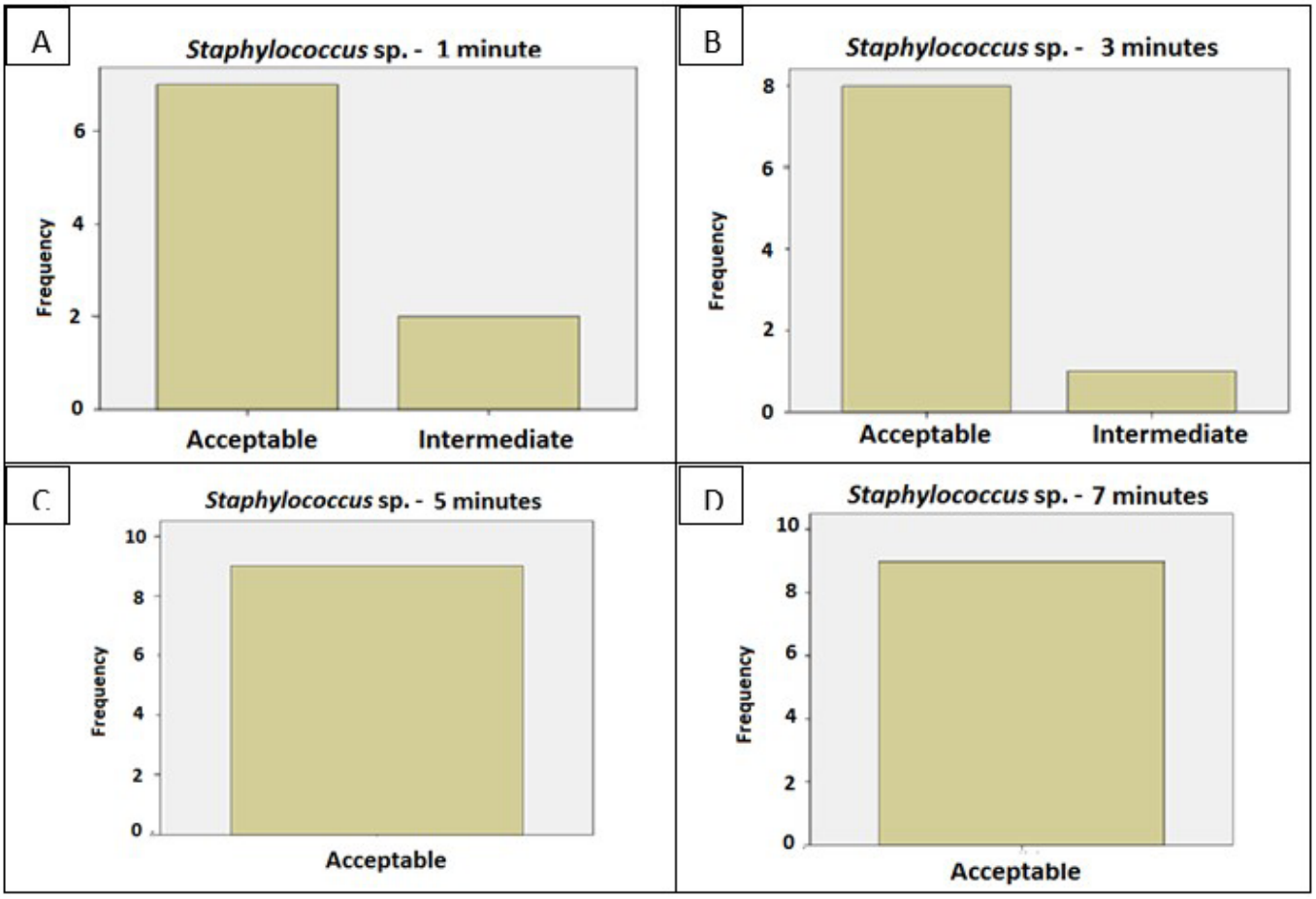

Figure 2. Judgment of mussel samples based on the count of coagulase-positive *Staphylococci* after thermal treatment for 1 minute (A), 3 minutes (B), 5 minutes (C) and 7 minutes (D), according to IN 60 (Brasil, 2019).

desirable to improve the quality, in order to include all the bivalves classified as acceptable according to the legislation.

After five and seven minutes, 100% (9) of the samples were acceptable, with values lower than 100 CFU/g. Thus, thermal treatment at 95°C from 5 (five) minutes ensures a safe product regarding the presence of coagulase-positive *Staphylococc*i.

The number of total coliforms ranged from 9 x 10 to 4.6 x 10^4 MPN/g in fresh mussels. In precooked mussels (control), it ranged from < 3 to 1.1 x 10^4 MPN/g. A significant difference ($p < 0.001$) was found between the time intervals evaluated considering this count. However, it was merely found that coliform counts in fresh mussels differed from those observed after at least three minutes of cooking. Although high, the number of total coliforms is not a parameter in Brazilian legislation. However, coliforms are considered an indicator of hygiene conditions in food processing: a high number of coliforms is associated with poor handling practices.

The judgment of fresh and precooked (control) mussels based on IN 60 (Brasil, 2019) according to the Most Probable Number (MPN) of *E. coli* is described in Figure 3.

For fish not consumed raw, *E. coli* parameters range from 50 to 5x10^2 MPN/g according to the Brazilian legislation (Brasil, 2019). Thus, in fresh mussels, only one sample (11.1%) was acceptable. The others (88.9%) had intermediate quality, ranging from 9 x 10 to 4.3 x 10^2 MPN/g. A significant difference ($p < 0.001$) was found between the time intervals evaluated regarding *E. coli* counts. However, although higher counts were noticed in fresh and peeled products, no significant differences were detected between the different time intervals in multiple comparisons (comparisons of 2 groups).

Less significant figures were described by Silva et al. (2021) in their evaluation of the microbiological quality of mussels *Perna perna* cultivated in Píuma, Espírito Santo. The authors found that the amount of total coliforms ranged from 28 to >1,600 MPN/g and the number of thermotolerant coliforms ranged from <2 to 21.5 MPN/g.

However, for Charru mussels extracted from Sepetiba Bay, Palmeira et al. (2018) reported a higher count of thermotolerant coliforms. A count of 3 x 10^5 MPN/g was found for fresh Charru mussels and 7.5 x 10^3 MPN/g for precooked Charru mussels, above the limits established by the legislation.

In the control group (precooked mussels), only one sample had intermediate quality (11.1%) regarding *E. coli* and the others (88.9%) were acceptable. Such finding contrasts with those of Leôncio et al. (2020) in São Luís (MA) who detected

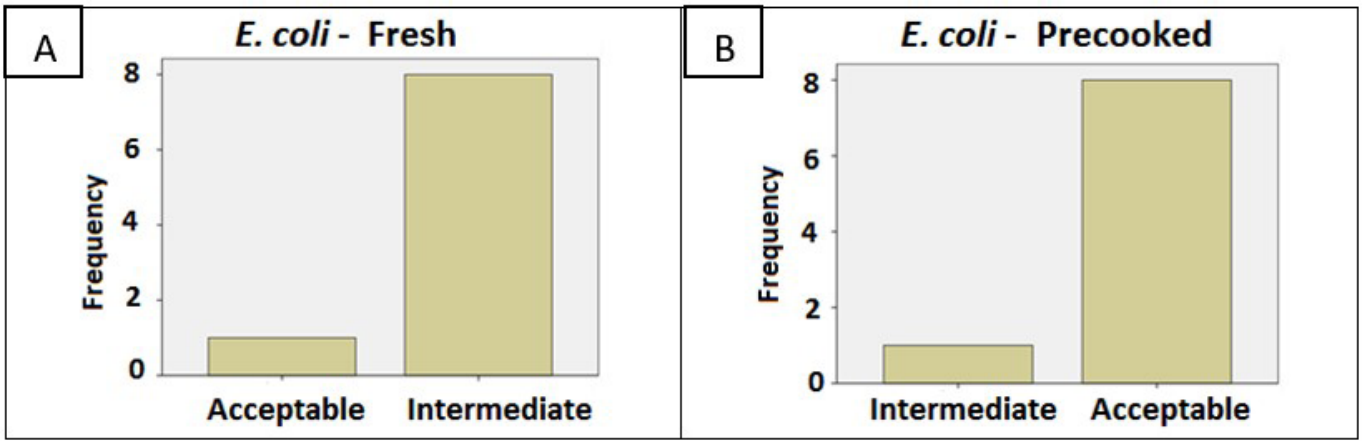

Figure 3. Results of the judgment of fresh (A) and precooked (B) mussels *Perna perna*, according to IN 60 (Brasil, 2019) based on *E. coli* MPN.

the presence of *E. coli* in 50% of the samples of Charru mussels, values above the levels acceptable by the legislation, suggesting poor conditions of preparation, conservation and marketing at room temperature.

In Maceió, Alagoas, Silva et al. (2015) found total and thermotolerant coliform counts above 1100 MPN/g in samples of Charru mussels, which may be related to the selling of the products in outdoor markets without meeting the minimum hygiene standards. In the present study, the precooked product (control) had the lowest *E. coli* count, with MPN ranging from < 3 to 9 x 10 MPN/g. The values found can be associated with the type of display of the product, sold in plastic packaging, and therefore not fully exposed during selling.

All thermal treatments at 95 °C for 1 (one) minute or longer were efficient to reduce *E. coli* counts to less than 3 MPN/g, making these foods acceptable to be consumed according to the Brazilian legislation (Brasil, 2019).

Araújo et al. (2009) also reported the efficiency of the thermal treatment when they observed the absence of *E. coli* in precooked Charru mussels from the village of Taiçoca de Fora, Sergipe. On the other hand, pathogens were found in fresh Charru mussels.

According to Nascimento et al. (2011), precooking of bivalve molluscs by steam or by immersion in water at a temperature of 95 °C for 5, 10 and 15 minutes is considered an efficient thermal treatment. The authors reported that no *Salmonella* spp was detected after these treatments, total thermotolerant coliforms were less than 3.0 MPN/g and CAMHB was less than 10 CFU/g, which is consistent with the present study where total coliforms and *E. coli* counts were reduced to less than 3 MPN/g after thermal treatment.

Salmonella spp was not found in any sample of the analyzed products. Guimarães et al. (2019) developed fish sausages from Brazilian flathead (*Percophis brasiliensis*) captured in Niterói City and also verified that according to Brazilian legislation they had absence of this bacteria in the analyzed products.

However, different results in the microbiological analysis for bivalve molluscs were found by others authors. In California, Shapiro et al. (2018) described contamination of mussels (*Mytilus californianus*) by serovars Enteritidis and Infantis. In Baía de Santos, Passos et. al. (2011) detected the presence of *Salmonella* spp. in 20.7% of the samples of mussels *Perna perna* analyzed at different times of the year. The dominant serotype was S. Livingstone, and other serotypes were also isolated, such as *S.* Infantis, *S. enterica* subsp. *salamae* and *S.* Albany.

Despite the presence of other enterobacteria, such as E. *coli* and the absence of *Salmonella* spp. in mussels, Freitas et al. (2015) reported that water contamination by total coliforms and *E. coli* may not be sufficient to contaminate bivalves.

As for the antibiotic resistance index of the detected bacteria, susceptibility of *E. coli* to different antimicrobials is described in Table 2.

Of the *E. coli* strains isolated from mussels, 58.34% were resistant to ampicillin. This result was expected, as according to Dias et al. (2010), *E. coli* is generally able to inactivate antibiotics of the penicillin group due to the action of β-lactamase enzymes.

In their evaluation of *E. coli* strains isolated from mussels *P. perna*, also in Niterói, Dias et al. (2010) found that only 2.27% of the strains were resistant to ampicillin, a percentage lower than that found in this study. However, a similar result was obtained by Vieira et al. (2008), who found that 50% of the *Crassostrea rhizophorae* oysters analyzed were resistant to ampicillin.

Resistance to cephalexin was found in 41.67% of the strains and to cefuroxime and norfloxacin, in 25%. Oliveira (2016) isolated *E. coli* strains from mussels collected in Santos and found a similar percentage (20%) of resistance to norfloxacin. Miotto et al. (2019) reported 96% of susceptibility to norfloxacin in bivalves cultivated in Santa Catarina, higher than in the present study where 75% of *E. coli* strains were susceptible to the antimicrobial.

Of the isolated strains, 8.34% were resistant to gentamicin, which is consistent with the findings of Jayme et al. (2014), who obtained 7.8% in the analysis of mussels isolated from Itaipu, Niterói.

The percentage of strains resistant to antimicrobials tetracycline and ciprofloxacin was 33.33%, higher than that reported by

Table 2. Susceptibility of *E. coli* to antimicrobials.

ANTIBIOTIC	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
TET	7 (58.34%)	1 (8.33%)	4 (33.33%)
GEN	11 (91.66%)	0 (0%)	1 (8.34%)
CRX	5 (41.67%)	4 (33.33%)	3 (25%)
AMP	4 (33.33%)	1 (8.33%)	7 (58.34%)
CFE	7 (58.33%)	0 (0%)	5 (41.67%)
CIP	7 (58.33%)	1 (8.33%)	4 (33.33%)
NOR	9 (75%)	0 (0%)	3 (25%)
NIT	8 (66.67%)	0 (0%)	4 (33.33%)
CFL	2 (16.66%)	3 (25%)	7 (58.33%)
CLO	2 (16.67%)	0 (0%)	10 (83.33%)

Table 3. Susceptibility of coagulase-positive *Staphylococci* to antimicrobials.

ANTIBIOTIC	SUSCEPTIBLE	INTERMEDIATE	RESISTANT
TET	17 (77.27%)	3 (13.63%)	2 (9.09%)
ERI	5 (22.73%)	5 (22.73%)	12 (54.54%)
GEN	19 (86.36%)	2 (9.09%)	1 (4.54%)
CLI	12 (54.55%)	3 (13.64%)	7 (31.81%)
CLO	19 (86.36%)	1 (5.54%)	2 (9.09%)
CIP	15 (68.18%)	4 (18.18%)	3 (13.64%)
NOR	18 (81.82%)	2 (9.09%)	2 (9.09%)
NIT	17 (77.27%)	1 (4.55%)	4 (18.18%)

Jayme et al. (2014), who found 10.3% resistance to tetracycline and 1.3% to ciprofloxacin. Vieira et al. (2008) found similar percentages for tetracycline in oysters, with 25% resistance of the strains, but no resistance of *E. coli* to ciprofloxacin was reported.

Although the high resistance to ciprofloxacin detected differed from that described by other authors, according to Oliveira (2016), the resistance profiles in *E. coli* obtained from aquatic environments vary significantly. One explanation is the impact of effluent emissions directly into rivers and seas, contaminating waters and conveying resistant microorganisms that can pose health risks.

This is consistent with the study of Vieira et al. (2008) who despite obtaining susceptibility in 100% of *E. coli* samples isolated from oysters to ciprofloxacin, it did not occur in water. The authors found resistance in 8% of the isolates from water.

There was also resistance of 33.33% of the strains to nitrofurantoin, higher than that of Miotto et al. (2019) who found 12% of resistant strains.

A percentage of 58.33% of resistance was found for cephalotin. This is similar to the findings of Rodrigues (2019) who assessed the quality of a lake in an urban perimeter and detected a resistance of 51% of *E. coli* strains. A lower percentage was found by Evangelista-Barreto et al. (2012), who reported 12.5% of the strains as resistant, although 62.5% were classified as having intermediate resistance.

Resistance to chloramphenicol was 83.33%, higher than the results of other authors. Rodrigues (2019) reported only 12% resistance, and Evangelista-Barreto et al. (2012) did not report *E. coli* resistance to chloramphenicol in fish marketed in Cruz das Almas, Bahia.

The results concerning coagulase-positive *Staphylococci* are described in Table 3.

Most antimicrobials were effective, with 86.36% of the strains susceptible to gentamicin and chloramphenicol; 81.82% to norfloxacin; 77.27% to tetracycline and nitrofurantoin; 68.18% to ciprofloxacin and 54.55% to clindamycin. Costa et al. (2008) found a resistance of 18.18% to tetracycline, similar to this study (9.09%).

All isolates (100%) were susceptible to norfloxacin and 89% to ciprofloxacin in Voloski (2018) studies. Costa (2016) also found high susceptibility to ciprofloxacin, with 91.66% of susceptible strains. In the present study, the percentage was lower, with 81.82% of susceptibility to norfloxacin and 68.18% to ciprofloxacin.

Only 18.18% of the strains were resistant to nitrofurantoin, contrasting with Voloski (2018) regarding the resistance profile of *Staphylococcus aureus* in merluccid hake (*Merluccius merluccius*). The author found that 44% of the strains were resistant to nitrofurantoin.

Most strains were resistant to erythromycin (54.54%) strains), followed by clindamycin (31.81%). Rosa et al. (2021) found lower values for tilapia fillets, with 12% of strains resistant to erythromycin and 20% to clindamycin. However, in the analysis of antimicrobial susceptibility of bacteria isolated from Jundiá, Costa et al. (2008) found a higher resistance to erythromycin (27.27%).

Also, 18.18% of the strains were resistant to nitrofurantoin and 13.64% to ciprofloxacin. Low values were found for tetracycline, chloramphenicol and norfloxacin (9.09%). Chloramphenicol efficacy was 86.36%. A similar result was found by Costa et al. (2008): 81.82% susceptibility of strains to this antimicrobial.

The most efficient antimicrobial was gentamicin, with only 4.54% of resistance and 86.36% of susceptibility. Rosa et al. (2021) found slightly higher values for gentamicin, with 14% of resistant strains.

The high resistance of *Staphylococcus* spp. to antimicrobials was expected, given their high adaptability. This can be explained by several mechanisms, which include mutation (alteration of the antibiotic target site) or acquisition of resistance genes from other bacteria (mediated by plasmids and transposons) leading to destruction or inactivation of the antimicrobial (Cussolim et al., 2021).

The occurrence of multidrug-resistant *Staphylococcus* spp. is common not only in fish and shellfish. Keyvan et al. (2020) identified *Staphylococcus aureus* strains isolated from a milk tank and found resistance to most antimicrobials. Resistance to clindamycin was 67.9%.

Resistance of microorganisms is a growing concern. Of the pathogens investigated, 66% of *E. coli* strains and 34.78% of coagulase-positive *Staphylococcus* strains were resistant to multiple

drugs. According to Maestri et al. (2020), these pathogens are among the most frequent species in hospital-acquired infections due to their high adaptability and resistance to antimicrobials. The high rate of multiple drug-resistant strains poses a risk to public health, making treatment difficult and aggravating potentially curable clinical conditions.

4 Conclusions

Inadequate processing of mussels, disregarding hygiene and sanitary practices, is a source of contamination. Thus, the products must be cooked prior to consumption, to reduce contamination.

Although pathogenic microorganisms are eliminated only after 5 minutes of cooking, the binomial time x temperature (95 °C for 1 minute) was sufficient to reduce these microorganisms to acceptable levels according to Brazilian legislation, making it a safe food.

High resistance to multiple antimicrobials was found in the pathogens studied. Thus, there are various resistance profiles, especially in the aquatic environment.

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