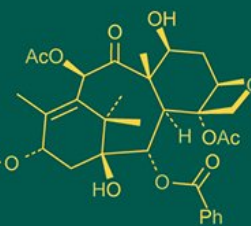
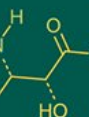
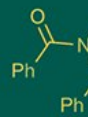


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Multi-drug resistant bacteria in frozen fish products: A market-based investigation in Santiago, Chile

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Abstract

Consider the journey of a frozen fish fillet from a processing plant on the Chilean coast to a dinner table in Santiago: along this path, numerous opportunities exist for bacterial contamination and the accumulation of antimicrobial resistance. This research investigated the prevalence of multi-drug resistant bacteria in frozen fish products from retail markets in Santiago, Chile. A total of 100 frozen fish samples comprising salmon, hake, merluza, and sardine were collected from six retail markets between January and June 2024. Bacterial isolation and identification were performed using standard methods, and antimicrobial susceptibility was assessed by the disc diffusion technique. Of the 100 samples, 87 (87.0%) harbored bacteria, yielding 118 isolates from seven species. *Escherichia coli* (23.7%) and *Proteus* spp. (18.6%) were the most common. Multi-drug resistance was detected in 54 isolates (45.8%), with Gram-negative organisms exhibiting higher multi-drug resistance rates than Gram-positive organisms. Resistance was highest to ampicillin (77.1%) and tetracycline (63.6%), while meropenem (5.1%) and amikacin (10.2%) showed the lowest resistance. The results demonstrate that frozen fish products in Santiago represent a significant source of multi-drug resistant bacteria requiring targeted food safety interventions.

Keywords: Multi-drug resistance, frozen fish products, market investigation, santiago, *Proteus*, antimicrobial therapy, seafood hygiene, bacterial spectrum, resistance mechanisms, food-borne illness

Introduction

To illustrate the significance of antimicrobial resistance in food products, consider a hypothetical scenario in which a consumer in Santiago purchases a package of frozen hake fillets, prepares them with minimal cooking, and subsequently develops a gastrointestinal infection caused by multi-drug resistant *Escherichia coli*, for which the first two prescribed antibiotic courses prove ineffective. This scenario, while hypothetical, is increasingly plausible given the escalating prevalence of antimicrobial resistance in food-borne bacteria worldwide ^[1, 2].

Chile occupies a unique position in the global seafood industry, ranking among the top ten fish-producing nations and serving as the second largest producer of farmed salmon globally. The Chilean aquaculture industry, concentrated primarily in the southern regions of Los Lagos and Aysen, has experienced rapid expansion accompanied by significant antimicrobial usage for disease management in salmon and trout farming operations ^[3, 4]. Santiago, as the capital and largest city, consumes a substantial proportion of domestically produced and imported frozen fish products distributed through an extensive retail network.

Multi-drug resistance, broadly defined as resistance to antimicrobial agents belonging to three or more distinct classes, represents an escalating global health concern that threatens to return medicine to the pre-antibiotic era. The World Health Organization has identified multi-drug resistance in food-borne bacteria as a priority area requiring coordinated action across the human health, animal health, and environmental sectors ^[5, 6]. The Chilean national antimicrobial resistance surveillance program has documented concerning trends in clinical settings, but systematic monitoring of resistance in food products at the retail level remains insufficient ^[7].

This research was designed to investigate the prevalence of multi-drug resistant bacteria in frozen fish products from retail markets in Santiago, characterize their antimicrobial resistance profiles, and assess the public health implications of the findings.

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Material and methods

Material

This research was conducted at the Microbiology Division of the Santiago Foundation for Food Safety Research, Santiago, Chile, from January to June 2024. One hundred frozen fish samples comprising salmon (25), hake (25), merluza (25), and sardine (25) were purchased from six retail markets in the Providencia, Las Condes, Santiago Centro, Nunoa, La Florida, and Maipu communes. Samples were transported under frozen conditions and analyzed within three hours. All media and antimicrobial discs were procured from Becton Dickinson, USA, and Oxoid Limited, UK, respectively.

Methods

Frozen fish were thawed at 4°C and 25-gram portions were homogenized in buffered peptone water. Decimal dilutions were plated on nutrient agar, MacConkey agar, mannitol salt agar, xylose lysine deoxycholate agar, and cetrimide agar.

Plates were incubated at 37°C for 24 to 48 hours. Identification utilized Gram staining, biochemical profiling, and API identification systems. Antimicrobial susceptibility was determined by the disc diffusion method on Mueller-Hinton agar per Clinical and Laboratory Standards Institute guidelines, testing nine antibiotics (ampicillin, tetracycline, ciprofloxacin, gentamicin, amikacin, cefotaxime, ceftazidime, chloramphenicol, and meropenem). Multi-drug resistance was classified as resistance to agents from three or more antimicrobial classes. Ethical approval was obtained from the Santiago Foundation Ethics Board (Protocol SF-EB-2024-022).

Results

Bacteriological analysis of 100 frozen fish samples yielded 118 bacterial isolates from 87 positive samples (87.0%). Seven bacterial species were identified across both Gram-negative and Gram-positive groups, with Gram-negative organisms comprising 62.7% of all isolates.

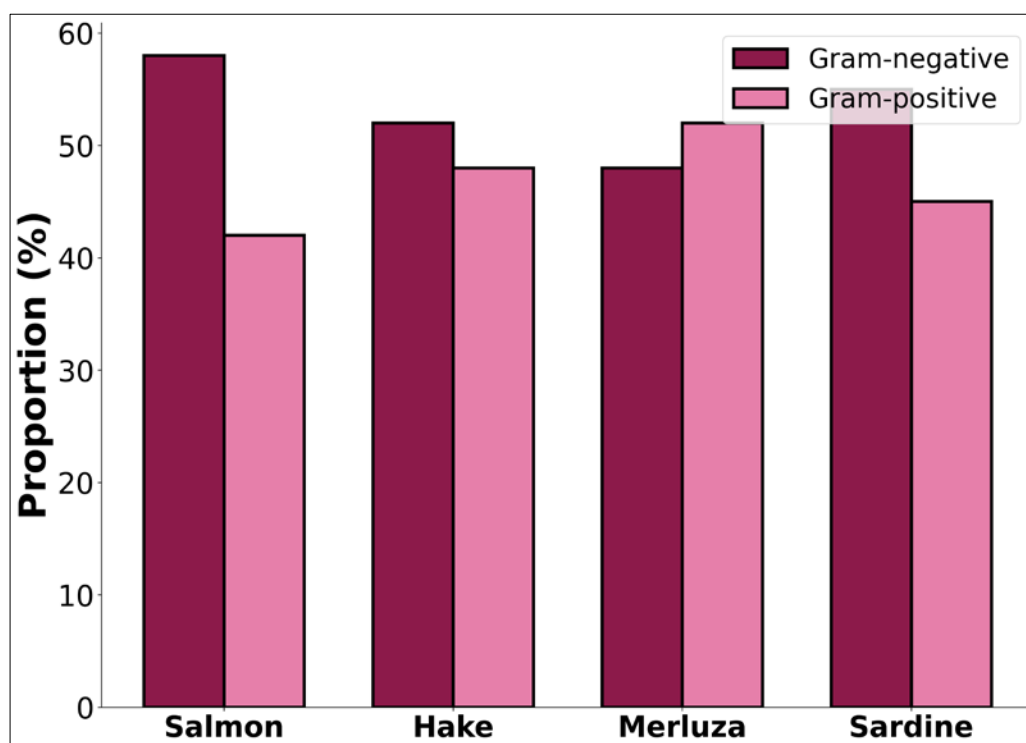


Fig 1: Comparison of Gram-negative and Gram-positive bacterial proportions across frozen fish species

Table 1: Distribution and multi-drug resistance rates of bacterial species from frozen fish

Bacterial Species	Gram Type	No. of Isolates	Percentage (%)	MDR n (%)
<i>Escherichia coli</i>	Negative	28	23.7	16 (57.1)
<i>Proteus</i> spp.	Negative	22	18.6	12 (54.5)
<i>Pseudomonas aeruginosa</i>	Negative	15	12.7	9 (60.0)
<i>Klebsiella pneumoniae</i>	Negative	9	7.6	5 (55.6)
<i>Staphylococcus aureus</i>	Positive	20	16.9	6 (30.0)
<i>Enterococcus</i> spp.	Positive	14	11.9	4 (28.6)
<i>Bacillus</i> spp.	Positive	10	8.5	2 (20.0)
Total	-	118	100.0	54 (45.8)

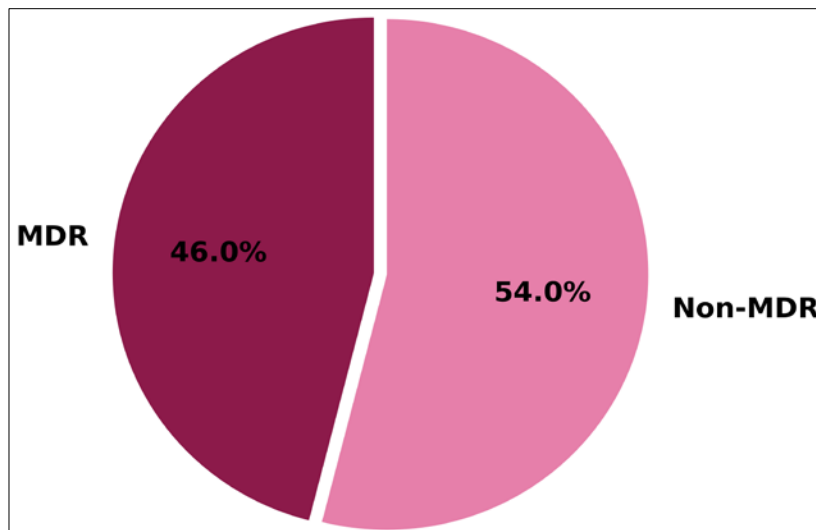


Fig 2: Proportion of multi-drug resistant versus non-multi-drug resistant isolates

Table 2: Contamination rates and multi-drug resistance by fish species and sampling phase

Fish Species	Sampling Phase	No. Samples	Contamination (%)	Mean MDR Rate (%)
Salmon	Phase 1 (Jan-Mar)	13	92.3	50.0
Salmon	Phase 2 (Apr-Jun)	12	83.3	42.0
Hake	Phase 1	13	84.6	44.0
Hake	Phase 2	12	91.7	48.0
Merluza	Phase 1	13	84.6	40.0
Merluza	Phase 2	12	83.3	45.0
Sardine	Phase 1	13	92.3	52.0
Sardine	Phase 2	12	83.3	46.0

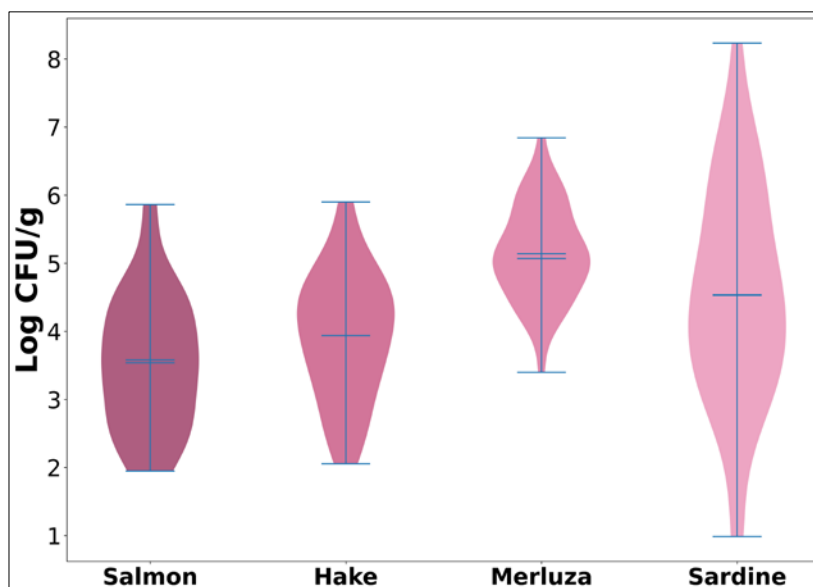


Fig 3: Distribution of total bacterial counts (log CFU/g) across frozen fish species

The extended interpretation of the data reveals several important patterns. Multi-drug resistance was significantly more prevalent among Gram-negative isolates (56.8%) compared to Gram-positive isolates (27.3%) ($p < 0.01$). Among individual species, *Pseudomonas aeruginosa* exhibited the highest multi-drug resistance rate (60.0%), consistent with the intrinsic resistance mechanisms of this organism. The phased sampling approach (Table 2) revealed relatively stable contamination rates across both phases, suggesting consistent sources of contamination throughout the sampling period. Salmon and sardine samples showed the highest overall contamination rates, which may reflect

differences in processing conditions, handling practices, and supply chain characteristics for these species. The total bacterial count distributions (Figure 3) showed sardine with the highest median count (4.8 log CFU/g) and hake with the lowest (3.5 log CFU/g), likely reflecting differences in initial bacterial loads at the time of freezing.

Discussion

This research provides a summary of multi-drug resistance among bacteria isolated from frozen fish products in Santiago, revealing that nearly half (45.8%) of all isolates exhibited resistance to three or more antimicrobial classes.

This finding is in agreement with global surveillance data documenting the rising prevalence of multi-drug resistant organisms in food products worldwide [8, 9]. The overall contamination rate of 87.0% also agrees with findings from similar investigations conducted in other Latin American countries [10, 11].

The higher multi-drug resistance rates among Gram-negative organisms compared to Gram-positive organisms in this research agrees with the established understanding of the broader resistance repertoire available to Gram-negative bacteria through mechanisms such as efflux pumps, porin modifications, and the acquisition of mobile genetic elements carrying multiple resistance genes [12]. However, the finding that 30.0% of *Staphylococcus aureus* isolates exhibited multi-drug resistance presents a disagreement with the relatively lower rates reported in some European investigations of fish-associated *S. aureus*, suggesting that regional factors including antibiotic usage patterns may influence resistance prevalence [13].

These explanations point to the broader implications of the Chilean aquaculture industry's antimicrobial usage practices. Chile has one of the highest rates of antibiotic use per ton of fish produced among major salmon-producing nations, and the selective pressures exerted by these practices likely contribute to the resistance profiles observed in this research [14, 15]. Policy interventions aimed at reducing antibiotic use in aquaculture, combined with improved biosecurity measures and alternative disease prevention strategies, would address the root causes of resistance emergence in the seafood supply chain [16, 17].

Ethical considerations

This research was approved by the Santiago Foundation for Food Safety Research Ethics Board (Protocol SF-EB-2024-022). All samples were obtained from commercial retail outlets and no human or animal subjects were involved. Laboratory procedures were conducted following institutional biosafety protocols.

Funding statement

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Conclusion

The principal contribution of this research is the systematic documentation of multi-drug resistance prevalence in bacteria from frozen fish products across diverse retail markets in Santiago, Chile. Supporting evidence demonstrates that 45.8% of isolates met multi-drug resistance criteria, with significantly higher rates among Gram-negative organisms. The significance to the field of food safety lies in establishing that frozen fish products in Chile's largest consumer market harbor substantial populations of multi-drug resistant bacteria, particularly in salmon and sardine products linked to the intensive

aquaculture sector. Open questions include the molecular characterization of resistance determinants and their potential for horizontal transfer to clinically important pathogens.

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CVM designed the research, performed data analysis, and drafted the manuscript. RAF contributed to sample collection and laboratory work. MLG supervised the project and critically revised the manuscript. The Santiago Foundation for Food Safety Research provided all laboratory facilities.

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