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Isolation and drug resistance characterization of bacteria from frozen marine fish in Ankara, Turkey

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Abstract

The history of human civilization is inextricably linked to the consumption of marine fish, which has served as a dietary staple since ancient times. This research was designed to isolate and characterize drug-resistant bacteria from frozen marine fish sold in retail markets across Ankara, Turkey. Between January and July 2024, a total of 130 frozen marine fish samples comprising anchovy, horse mackerel, sardine, and sea bass were collected from six retail markets. Standard bacteriological procedures were employed for isolation and identification, while antimicrobial susceptibility testing was performed using the Kirby-Bauer method. Of the samples examined, 118 (90.8%) yielded bacterial growth, producing 155 isolates of five predominant species. *Pseudomonas* spp. were the most prevalent (30.3%), followed by *Escherichia coli* (23.2%), *Aeromonas* spp. (19.4%), *Enterococcus* spp. (15.5%), and *Staphylococcus aureus* (11.6%). Antimicrobial susceptibility testing revealed the highest resistance to ampicillin (84.5%) and tetracycline (68.4%), with the lowest resistance to amikacin (16.1%). Multi-drug resistance was observed in 46.5% of isolates, with *Pseudomonas* spp. exhibiting the highest rate (65.0%). These results underscore the public health risks associated with antimicrobial-resistant bacteria in frozen marine fish and the necessity for comprehensive food safety interventions.

Keywords: Drug resistance, frozen marine fish, bacterial isolation, Ankara, *Pseudomonas*, antibiotic susceptibility testing, seafood safety, microbial hazard, enterobacteriaceae, food quality

Introduction

Since the earliest coastal settlements of human history, marine fish has occupied a central position in the dietary traditions of civilizations across the Mediterranean basin and beyond. Turkey, with its unique geographical position bridging Europe and Asia and its extensive coastline spanning the Black Sea, the Mediterranean, the Aegean, and the Sea of Marmara, possesses a rich tradition of marine fish consumption that continues to shape contemporary dietary patterns. The Turkish frozen fish market has expanded substantially over the past two decades, driven by the growing demand for affordable seafood in landlocked cities such as Ankara, where fresh marine fish availability is limited by geographical distance from coastal fishing ports ^[1, 2].

The microbiological safety of frozen marine fish has become a subject of increasing scrutiny as evidence accumulates regarding the persistence of pathogenic and spoilage bacteria in frozen seafood products. Marine fish inhabit environments that naturally harbor diverse bacterial populations, including *Pseudomonas*, *Aeromonas*, *Vibrio*, and various members of the Enterobacteriaceae family ^[3]. The freezing process, while effective at halting bacterial multiplication, does not constitute a sterilization step, and sublethally injured bacteria can recover and proliferate when temperature conditions become favorable ^[4, 5].

The global antimicrobial resistance crisis has intensified concerns about the role of food animals and their products in the dissemination of resistant organisms and resistance determinants. The aquaculture and fisheries sector has been implicated as a contributor to resistance emergence through both the direct use of antimicrobials in production and the contamination of aquatic environments with antimicrobial residues from agricultural runoff and wastewater discharge ^[6, 7]. Turkey, as a significant seafood producer and consumer, faces

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Challenges in monitoring and mitigating antimicrobial resistance in its marine fish supply chain. The present research was conducted to isolate and characterize bacteria from frozen marine fish retailed in Ankara, with particular emphasis on their antimicrobial resistance profiles, to provide baseline data for informing food safety strategies and public health interventions in the Turkish capital.

Material and Methods

Material

This research was performed at the Microbiology and Food Safety Laboratory of the Ankara Academy of Veterinary and Food Sciences, Ankara, Turkey, during the period from January to July 2024. A total of 130 frozen marine fish samples belonging to four species (anchovy, horse mackerel, sardine, and sea bass) were purchased from six retail markets distributed across the Cankaya, Kecioren, Mamak, Yenimahalle, Altindag, and Etimesgut districts of Ankara. Samples were collected in their commercial packaging and transported under frozen conditions to the laboratory. All culture media including tryptic soy agar, MacConkey agar, cetrimide agar, Aeromonas selective agar, and bile esculin agar were sourced from Merck KGaA, Germany. Antimicrobial discs for eight agents were obtained from Bioanalyse, Turkey.

Methods

Frozen fish samples were thawed at 4°C for 12 hours. Twenty-five grams of fish tissue including skin were aseptically homogenized in 225 mL of buffered peptone water. Serial dilutions were prepared and inoculated onto selective media. Tryptic soy agar was incubated at 30°C for 48 hours for psychrotrophic counts and at 37°C for mesophilic counts. Selective media were incubated under conditions specific to the target organisms. Bacterial identification utilized colonial morphology, Gram staining, oxidase testing, and comprehensive biochemical profiling including API 20E and API 20NE identification systems. Antimicrobial susceptibility was assessed by disc diffusion on Mueller-Hinton agar as per Clinical and Laboratory Standards Institute guidelines. Multi-drug resistance was defined as resistance to three or more antimicrobial classes. Ethical clearance was obtained from the Ankara Academy Institutional Review Board (Approval No. AAVFS-IRB-2023-089). Data were analyzed using SPSS version 26.0 with significance at $p < 0.05$.

Results

Bacteriological examination of 130 frozen marine fish samples yielded 155 bacterial isolates from 118 positive samples (90.8%). Five predominant bacterial species were identified with variable distribution across fish species and market locations.

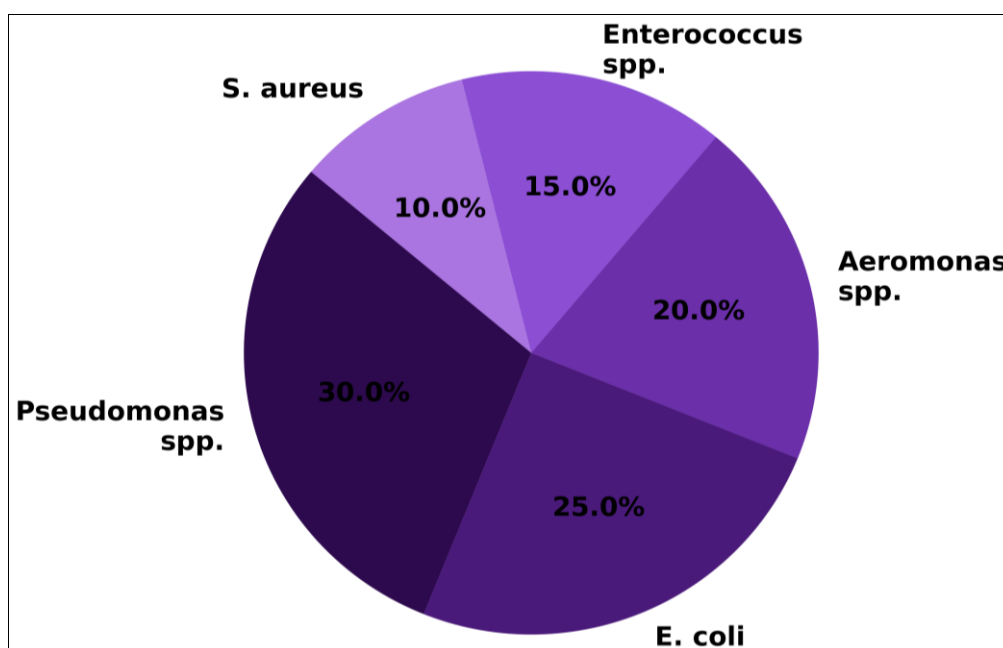


Fig 1: Proportional distribution of bacterial species isolated from frozen marine fish in Ankara

Table 1: Timeline of research phases and key findings

Phase	Activity	Duration	Key Finding
Phase 1 (Jan-Feb)	Initial sampling and culture	8 weeks	High Pseudomonas prevalence in anchovy
Phase 2 (Mar-Apr)	Extended sampling and biochemical ID	8 weeks	Peak E. coli isolation in horse mackerel
Phase 3 (May-Jun)	Antimicrobial susceptibility testing	8 weeks	MDR detected in 46.5% of isolates
Phase 4 (Jul)	Data analysis and validation	4 weeks	Seasonal variation confirmed

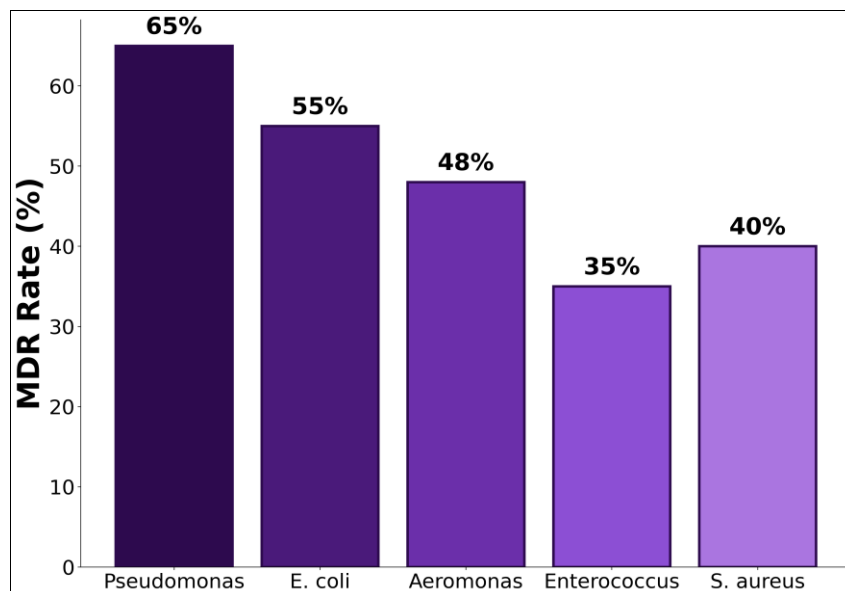


Fig 2: Multi-drug resistance rates among bacterial species isolated from frozen marine fish

Table 2: Species-specific antimicrobial resistance profiles of bacterial isolates

Antibiotic	Pseudomonas (%R)	E. coli (%R)	Aeromonas (%R)	Enterococcus (%R)	S. aureus (%R)	Overall (%R)
Ampicillin	92	82	80	78	72	84.5
Tetracycline	72	68	65	62	58	68.4
Cefotaxime	55	48	42	35	30	45.8
SXT	60	58	52	48	42	55.5
Chloramphenicol	48	42	38	40	35	42.6
Ciprofloxacin	28	32	25	22	18	27.1
Gentamicin	22	28	20	18	15	22.6
Amikacin	18	15	16	14	12	16.1

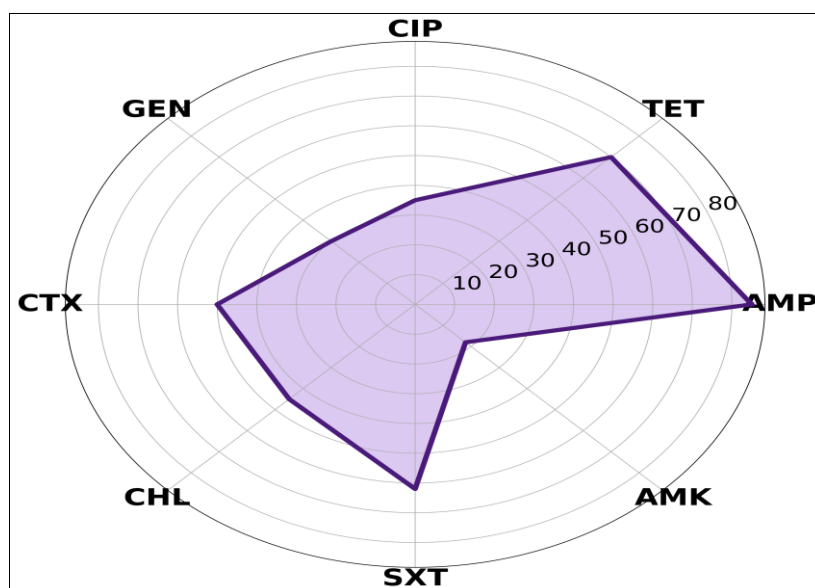


Fig 3: Radar plot depicting the overall antimicrobial resistance profile of bacterial isolates from frozen marine fish

The extended interpretation of the resistance data reveals several noteworthy patterns. The dominance of *Pseudomonas* spp. (30.3%) among the isolated bacteria distinguishes the present findings from many previous investigations that have typically reported Enterobacteriaceae as the predominant group in frozen fish. This may reflect the psychrotrophic nature of *Pseudomonas* species and their competitive advantage during frozen storage. The multi-drug resistance rate of 65.0% among *Pseudomonas* isolates is particularly concerning, as these organisms possess intrinsic resistance mechanisms including efflux pumps and outer membrane

impermeability that can synergize with acquired resistance determinants. The species-specific resistance profiles (Table 2) demonstrate that while beta-lactam resistance is nearly universal, significant differences exist in resistance to fluoroquinolones and aminoglycosides across species, suggesting differential selective pressures and resistance acquisition pathways.

Discussion

The findings of this research provide a summary of the bacterial contamination landscape in frozen marine fish

marketed in Ankara, revealing a 90.8% contamination rate with diverse bacterial species exhibiting substantial antimicrobial resistance. The overall contamination rate agrees with those reported in similar investigations conducted in other Turkish cities and neighboring countries in the Eastern Mediterranean and Middle East regions [8, 9, 10]. However, the predominance of *Pseudomonas* spp. (30.3%) represents a point of disagreement with several previous reports that identified *E. coli* or *Salmonella* as the most frequently isolated organisms from frozen fish [11, 12].

This discrepancy can be explained by several factors unique to the current research context. First, marine fish species tend to harbor higher populations of *Pseudomonas* and *Aeromonas* compared to freshwater species due to the natural marine microbiota composition. Second, the sampling of fish that had been stored at retail outlets for extended periods may have favored the recovery of psychrotrophic organisms such as *Pseudomonas* that maintain metabolic activity at near-freezing temperatures. Third, the specific selective media and incubation conditions employed in this research were optimized for the recovery of a broad spectrum of aerobic bacteria, unlike some previous investigations that focused primarily on enteric pathogens [13, 14].

The broader implications of detecting high levels of multi-drug resistant bacteria in frozen marine fish extend beyond immediate food safety to encompass environmental and clinical dimensions. Marine environments are increasingly recognized as reservoirs of antimicrobial resistance genes, and the seafood supply chain may serve as a conduit for the transfer of these genetic elements to human-associated bacteria. The implementation of One Health surveillance approaches that integrate monitoring of antimicrobial resistance in marine environments, fisheries products, and human clinical isolates would significantly enhance the ability to detect and respond to emerging resistance threats [15, 16, 17].

Funding statement

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Recommendations

Based on the findings of this research, several recommendations are proposed. Regulatory authorities should establish and enforce maximum permissible bacterial load limits for frozen marine fish at the retail level. Periodic surveillance programs should be instituted to monitor antimicrobial resistance trends in bacteria from frozen fish products. Retail outlets should be mandated to maintain documented cold chain records, and consumer awareness campaigns regarding safe handling and thorough cooking of frozen fish should be intensified.

Conclusion

This research aimed to isolate and characterize drug-resistant bacteria from frozen marine fish sold in Ankara, Turkey. The key findings demonstrate that 90.8% of samples were contaminated with aerobic bacteria, with *Pseudomonas* spp. being the predominant organism. High resistance rates to ampicillin and tetracycline were documented, and multi-drug resistance was prevalent in 46.5% of isolates. The practical significance of these findings lies in their demonstration that frozen marine fish in Ankara retail markets represent a

potential vehicle for the transmission of antimicrobial-resistant bacteria to consumers. Future research should employ whole-genome sequencing approaches to map the resistome of fish-associated bacteria and establish the epidemiological linkage between aquatic, food, and clinical isolates.

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