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Research Article

Phenotypic and Genotypic Antibiotic Resistance of Bacteria Isolated from Ready-to-eat Salted Seafood

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ABSTRACT

Safe food production faces significant challenges from both disease-causing bacteria and antibiotic-resistant bacteria, as both pose serious risks to public health and food safety. This study investigated the presence of antibiotic-resistant bacteria in salted seafood (lakerda) samples obtained from fishermen and fish markets. Phenotypic analysis revealed that lakerda samples collected from fishermen contained bacteria with multi-antibiotic resistance, including *Pseudomonas fluorescens, Staphylococcus haemolyticus,* and *Staphylococcus equorum*. *Carnobacterium maltaromaticum*, *Carnobacterium mobile,* and *Vibrio hibernica* species were isolated in larkerda samples sold by fish markets. It was determined that among isolated bacteria, *V. rumoiensis* did not contain any of the genotypically tested genes. However, *P. fluorescens* carried *blaTEM, qnrB, qnrS, blaZ,* and *msrA*; *S. haemolyticus* harbored *blaTEM, tetK, dfrD, blaZ, msrA, msrB,* and *mecA*; *C. maltaromaticum* possessed *blaTEM, qnrA, qnrB, qnrS, strA-strB, aphAI-IAB*, and *mecA*; *C. mobile* included *blaTEM, blaZ, msrA, dfrD,* and *mecA*; and *V. hibernica* carried *blaTEM, blaZ, mecA,* and *VanA*. In addition, *S. pasteuri* and *S. equorum* had the *mecA* resistance gene. In conclusion, public health needs to provide hygiene conditions in the preparation of lakerda, determine the ways of transmission, take precautions, and raise awareness of producers and consumers.

Keywords: Food safety, lakerda, antibiotic resistance, resistance genes, microbiology

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INTRODUCTION

Food selection is of the utmost importance in increasing the number of healthy individuals and making future generations healthier. For this reason, consuming high-quality and safe food should be the priority. In addition to the treatment costs of diseases caused by malnutrition, the fact that individuals are out of production during the disease processes harms the country's economy. In developed countries, governments pursue policies on the importance of food, the sustainability of food resources, and the delivery of safe food to consumers (Koç & Uzmay, 2015).

Antibiotic resistance, a critical issue, is the condition when antibiotics, crucial in treating or preventing bacterial diseases, lose their effectiveness in killing bacteria or halting their reproduction (World Health Organization, 2017). The escalating causes of antibiotic resistance, including the indiscriminate use, poor dosage, and overuse of antibiotics in livestock and agriculture, are alarming. Bacteria in the natural flora, indicator bacteria, and pathogenic bacteria are acquiring resistance to various antibiotics. Moreover, the rise in antibiotic resistance increases the risk of transferring antibiotic-resistance genes from bacteria in the natural flora to pathogenic bacteria (Urban-Chmiel et al., 2022). This urgent issue demands our immediate attention and action.

The bacterium's genetic material can be transferred to another bacterium of the same species or even to bacteria of different species.

Thus, bacteria can show multiple antibiotic resistance (Sharma et al., 2014). As a result, the antibiotics used to treat diseases cannot be utilized as targeted. As reported in the Global Action Plan report prepared in England, it is projected that bacteria resistant to antibiotics will become strong enough to kill one person every three seconds in the world by 2050. The same report also stated that the fight against resistant bacteria will be worth 100 trillion USD (O'Neill, 2016). In Türkiye, an estimated 7,607 deaths in 2021 were directly attributable to bacterial antimicrobial resistance, while 30,490 deaths were associated with infections where resistance played a contributing role (Measuring Infectious Causes and Resistance Outcomes for Burden Estimation, 2024).Salting is one of the oldest methods of food preservation. The practice of salting fish was carried out in the ancient Egyptian civilization for the first time. Salt is used in different stages of seafood storage. The purpose of salting applied to seafood is to bind the water in the product by the salt, thereby reducing water activity and thus preventing spoilage. The antiseptic effect of the chlorine in salt is also among the positive effects. Lakerda is a traditional product that uses the salting method in aquatic products and is mainly made from toric and bonito fish. The salting process, done initially to prevent the fish from spoiling, is now used to create a different flavor (Aksu et al., 2013). Technological applications (such as heat treatment or pressure application) that will inhibit the existing microorganism load to a large extent are not carried out during the production stage (Turan et al., 2009; Duyar et al., 2020). Before the presentation, salted foods are often consumed directly without any processing, such as cooking, to reduce or remove the microorganism load (Turan et al., 2009; Duyar et al., 2020).

Ensuring and preserving the health of individuals in society is a shared goal across all political views (Erbaydar, 2003). Monitoring and preventing microorganisms that could threaten human health in ready-to-eat foods is crucial. The present study is of utmost importance as it aims to identify the presence of antibiotic-resistant bacteria in lakerda products and investigate the carriers of resistance genes. This research is vital to monitoring antimicrobial resistance and evaluating its risks to human health.

MATERIALS AND METHODS

Materials

In the study, 30 samples of packaged lakerda, obtained from three different fish markets and three different fishermen in Türkiye, were used to isolate antibiotic-resistant bacterial isolates. Care was taken to ensure that commercial products were not close to their expiration dates and that the samples taken from the fishermen were lakerda prepared from the new fishing season. The samples were brought to the laboratory in styrofoam boxes (+ 4 °C). Lakerda samples were kept in the refrigerator (+ 4 °C) until microbiological analysis, which was conducted within a maximum of 7 days.

Bacterial isolations and identification

10 g of samples taken from lakerda samples for microbiological analysis were homogenized for one minute in 90 ml of peptone water. Decimal dilutions of the homogenate (10^{-1} to 10^{-6}) were then prepared. Inoculations were done from these dilutions according to the spreading and pour plate methods. The media were incubated at the appropriate temperature, and incubation times were shown in Table 1 for the growth of microorganisms in the incubator. Different types of colonies were developed on Tryptic Soy Agar (TSA), TSA+10% Sodium chloride (NaCl), and DeMan, Rogosa and Sharpe (MRS) media. Then, each isolate was inoculated separately in suitable liquid media. After the growth of bacteria on the medium, they were stored in sterile cryogenic tubes at -80°C in the presence of 30% glycerol.

Table 1. Media used for isolation of bacteria and incubation temperatures.

Parameters	Media	Temperature (°C)	Incubation time	Reference
Total heterotrophic viable bacteria	TSA	22°C	5 days	This study
Total halophilic bacteria	TSA +10% NaCl	30°C	10 days	Brillantes et al., 2002; Ruginescu et al., 2020
Lactobacillus spp. count	MRS	30°C	3-5 days	Jokovic et al., 2008

TSA: Tryptic Soy Agar, NaCl: Sodium Chloride, MRS: DeMan, Rogosa ve Sharpe Agar

Bacteria were defined as heterotrophic, halophilic, and lactic acid bacteria groups, according to the media in which they grow. Forty-one isolates were identified as *Pseudomonas* spp., 40 were identified as *Staphylococcus* spp., 36 were identified as *Carnobacterium* spp., ten identified as *Vibrio* spp., and five identified as *Lactobacillus* spp. by standard microbiological procedures. These procedures included analyses of colony type, color, morphology, gram +/- characteristics, oxidase, catalase, H_2S, indole, oxidation/fermentation, arginine dihydrolase, lysine decarboxylase, β galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, H2S, simmons citrate, nitrate reduction, sensitivity to: O/129, tolerance to salinity (NaCl), growth at different temperature ranges, pH ranges, acid production from various carbohydrates like mannitol, inulin, rhamnose, dulcitol, salicin, sorbitol, trehalose, lactose, sucrose, maltose, galactose, xylose, cellobiose, and raffinose In the next step, 16S rDNA gene sequencing analysis was performed to precisely determine the bacterial species resistant to three or more antibiotics detected due to the antibiogram test. DNA isolation was carried out using the EurX GeneMATRIX Bacterial & Yeast DNA Isolation Kit (Poland). The PCR reaction mixture (35 µL) contained 1x PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 100 ng of template DNA, 0.3 µM of each primer, and nuclease-free water. PCR amplification was performed in a thermal cycler (Kyratec, Geumcheon-gu, Seoul, Korea) under the following conditions: an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 45 seconds (denaturation), 57°C for 45 seconds (annealing), and

72°C for 60 seconds (extension), with a final extension at 72°C for 5 minutes. A 10 µL aliquot of the PCR product was loaded onto a 1.5% agarose gel in 1x TAE buffer containing ethidium bromide, and electrophoresis was conducted for 90 minutes at 100 volts. The PCR products were subsequently purified and sequenced by BM Labosis (Ankara, Türkiye) using the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') (Lane, 1991). Sequence editing was performed using BioEdit software (BioEdit v7.0.0). The obtained 16S rRNA gene sequences were compared against the GenBank bacterial sequence database using the BLASTN tool available at http://blast.ncbi.nlm.nih.gov/.

Determination of antibiotic resistance

Kirby-Bauer's disc diffusion method (Bauer et al., 1966) was used to determine the antibiotic resistance of bacteria. Bacterial isolates were grown in Muller Hinton (MH) (1.5% saline and 10% saline) and MRS media. Afterward, they were transferred to solid media of the same growth medium. By choosing the colonies that show the best growth in the growth medium, density was adjusted to 0.5 McF in the broth medium. The transfer was made with a sterile cotton swab from the liquid broth medium with an adjusted density (0.5 McF) on the appropriate solid growth medium. Various antibiotic discs amoxicillin / clavulanic acid (30 µg), ampicillin (AMP) (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (10 µg), doxycycline hydrochlorıde (30 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), oxacillin (5 µg), streptomycin (10 µg), sulfamethoxazole/trimethoprim (25 µg), tetracycline (30 µg) and vancomycin (30 µg)] were placed in the medium at regular intervals using sterile forceps and left to incubate at the appropriate temperature and time (Table 1). The analyses were conducted with three repetitions. Zone diameters formed after incubation were measured. Afterward, the resistance or susceptibility of bacteria to antibiotics was evaluated according to the Clinical and Laboratory Standards Institute standards (CLSI, 2017), which provide interpretive criteria for classifying bacterial isolates as resistant, intermediate, or susceptible based on zone diameter measurements.

Identification of resistance genes of bacterial isolates

As a result of the antibiogram test, the presence of antibiotic-resistance genes in bacteria resistant to three or more antibiotics was investigated using a method previously applied in our laboratory (Kahraman Yilmaz and Berik 2024), briefly described below. Twenty-two antibiotic-resistant bacterial strains were screened for the presence of antibiotic resistance genes using uniplex PCR. The PCR reaction mixture (35 µL) consisted of 1x PCR buffer (Solis Biodyne), 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µM of each forward and reverse primer, 2 U Taq polymerase, the DNA template, and PCR-grade distilled water. The primer sequences used for amplification are listed in Table 2, while the PCR conditions for groups A, B, and C are detailed in the table footnote.

The PCR products were analyzed on a 1.0% agarose gel prepared in 1x TAE buffer. Electrophoresis was performed at 100 volts for 60 minutes, and the bands were visualized under UV light using ethidium bromide staining. The sizes of the amplicons were determined using 50 bp and 100 bp DNA size markers (Siripornmongcolchai et al., 2002).

Table 2. List of primers for detection of antimicrobial resistance genes.

Targeted gene		Sequence (5'-3')		References
β-lactamases	*bla*$_{TEM***}$	F	CATTTCCGTGTCGCCCTTATTC	Dallenne et al., 2010
		R	CGTTCATCCATAGTTGCCTGAC	
	bla$_{S}$*Hv****	F	AGCCGCTTGAGCAAATTAAAC	
		R	ATCCCGCAGATAAATCACCAC	
	bla$_{C}$*rx-M***	F	CGCTTTGCGATGTGCAG	Paterson et al., 2003
		R	ACCGCGATATCGTTGGT	
	*blaZ***	F	CAAAGATGATATAGTTGCTTATTCTCC	Kaase et al., 2008
		R	TGCTTGACCACTTTTATCAGC	
	*mecA**	F	GTGAAGATATACCAAGTGATT	Alfatemi et al., 2014
		R	ATGCGCTATAGATTGAAAGGAT	
	bla$_{IMI}$*p**	F	GAATAGAGTGGATTAATTCTC	Henriques et al., 2006
		R	GGTTTAAYAAAACAACCACC	
Tetracycline	*tetA****	F	GTAATTCTGAGCACTGTCGC	Sengeløv et al., 2003
		R	CTGCCTGGACAACATTGCTT	
	*tetB***	F	CTCAGTATTCCAAGCCTTTG	Sunde & Sørum, 2001
		R	CTAAGCACTTGTCTCCTGTT	
	*tetE****	F	GTGATGATGGCACTGGTCAT	Sengeløv et al., 2003
		R	CTCTGCTGTACATCGCTCTT	
	*tetK****	F	TATTTTGGCTTTGTATTCTTTCAT	Trzcinski et al., 2000
		R	GCTATACCTGTTCCCTCTGATAA	
	*tetM**	F	ACAGAAAGCTTATTATATAAC	Aminov et al. 2001
		R	TGGCGTGTCTATGATGTTCAC	

Table 2. Continue.

Targeted gene		Sequence (5'-3')		References
Chloramphenicol	*Cat A***	R	GGATATGAAATTTATCCCTC	Aarestrup, 2000
		F	CAATCATCTACCCTATGAAT	
	*Cat B***	R	TGAACACCTGGAACCGCAGAG	Xia et al., 2013
		F	GCCATAGTAAACACCGGAGCA	
Plasmid-mediated quinolone resistance	*qnrA***	F	AGAGGATTTCTCACGCCAGG	Cattoir et al., 2007
		R	TGCCAGGCACAGATCTTGAC	
	*qnrB***	F	GATCGTGAAAGCCAGAAAGG	Katalin, 2000
		R	ACGATGCCTGGTAGTTGTCC	
	*qnrS***	F	GCAAGTTCATTGAACAGGGT	Cattoir et al., 2007
		R	TCTAAACCGTCGAGTTCGGCG	
Aminoglycoside resistance	*strA-strB***	F	TATCTGCGATTGGACCCTCTG	Sunde & Sørum, 2001
		R	CATTGCTCATCATTTGATCGGCT	
	*aphAI-IAB***	F	AAACGTCTTGCTCGAGGC	Frana et al., 2001
		R	CAAACCGTTATTCATTCGTGA	
	*aac(3)-IIa***	F	ATGGGCATCATTCGCACA	Dai et al., 2010
		R	TCTCGGCTTGAACGAATTGT	
	*aac(6')-Ib***	F	TTGCGATGCTCTATGAGTGGCTA	Katalin, 2000
		R	CTCGAATGCCTGGCGTGTTT	
Vancomycin	*VanA***	F	GTACAATGCGGCCGTTA	Dutka-Malen et al., 1995
		R	GGGAAAACGACAATTGC	
	*VanB***	F	GTGCTGCGAGATACCACAGA	Ramos-Trujillo et al., 2003
		R	CGAACACCATGCAACATTTC	
Folate pathway inhibitors	*dfrD***	F	CCCTGCTATTAAAGCACC	Dale et al., 1995
		R	CATGACCAGATAACTC	
	*dfrK**	F	CAAGAGATAAGGGGTTCAGC	Argudín et al., 2011
		R	ACAGATACTTCGTTCCACTC	
	*dfrG***	F	TGCTGCGATGGATAAGAA	
		R	TGGGCAAATACCTCATTCC	
	*dfrA**	F	CACTTGTAATGGCACGGAAA	
		R	CGAATGTGTATGGTGGAAAG	
Macrolides	*ermA***	F	GTTCAAGAACAATCAATACAGAG	Lina et al., 1999
		R	GGATCAGGAAAAGGACATTTTAC	
	*ermB***	F	CCGTTTACGAAATTGGAACAGGTAAAGGGC	
		R	GAATCGAGACTTGAGTGTGC	
	*ermC***	F	GCTAATATTGTTTAAATCGTCAATTCC	
		R	GGATCAGGAAAAGGACATTTTAC	
Lincosamides	*msrA****	F	GGCACAATAAGAGTGTTTAAAGG	
		R	AAGTTATATCATGAATAGATTGTCCTGTT	
	*msrB***	F	TATGATATCCATAATAATTATCCAATC	
		R	AAGTTATATCATGAATAGATTGTCCTGTT	
	*lnuA***	F	GGTGGCTGGGGGGTAGATGTATTAACTGG	
		R	GCTTCTTTTGAAATACATGGTATTTTTCGA	
	*lnuB***	F	CCTACCTATTGTTTGTGGAA	Bozdogan et al., 1999
		R	ATAACGTTACTCTCCTATTC	

*PCR conditions group A: initial denaturation at 95 °C for 5 min, followed by 35 cycle consisting of denaturation at 95 °C for 40 s, 40 s annealing at 56 °C, 30 s extension at 72 °C, followed by a final extension step at 72 °C for 5 min.
**PCR conditions group B: initial denaturation at 95 °C for 5 min, followed by 35 cycle consisting of denaturation at 95 °C for 40 s, 40 s annealing at 56 °C, 45 s extension at 72 °C, followed by a final extension step at 72 °C for 5 min.
***PCR conditions group C: initial denaturation at 95 °C for 5 min, followed by 35 cycle consisting of denaturation at 95 °C for 40 s, 40 s annealing at 56 °C, 60 s extension at 72 °C, followed by a final extension step at 72 °C for 5 min.

RESULTS AND DISCUSSIONS

Detection of antibiotic-resistant bacteria in seafood is a major concern due to its implications for food safety and public health. In this study, multiple antibiotic-resistant bacteria, including *Pseudomonas fluorescens, Staphylococcus haemolyticus, Carnobacterium maltaromaticum* and *Carnobacterium mobile*, were identified in lakerda samples. The presence of resistance genes such as blaTEM, mecA and qnrB in these isolates highlights the potential of these bacteria to contribute to the spread of antimicrobial resistance. Such findings highlight the importance of monitoring ready-to-eat seafood, as they may serve as reservoirs for resistant bacteria and increase the risk of transmission of resistance genes to human pathogens. This issue is particularly critical for public health as it may lead to treatment failures and limited therapeutic options in bacterial infections.

Bacterial isolations, identifications, and antibiotic resistance

In this study, 132 bacteria were isolated from lakerda samples. Among these bacteria, 59.09% did not resist any of the 19 antibiotics tested, while 40.91% resisted at least one antibiotic. Among the bacterial isolates, 27.27% were resistant to three or more antibiotics (Figure 1). In addition, 40.74% of the isolates from commercial lakerda and 59.26% from fisherman-prepared lakerda exhibited resistance to at least one antibiotic. Among the bacterial isolates, resistance to antibiotics varied, with the highest resistance observed for NA (11.3%) and SXT (10.8%). This was followed by OX and CTX (both 7.9%), DA and S (both 7.4%), and CRO (6.4%). Additionally, resistance rates for other antibiotics were as follows: KF (5.9%), K (5.4%), E (4.9%), CN and VA (both 4.4%), AMP (3.9%), CIP (3.4%), AMC (3.0%), C (2.5%), and DO, TE, and IPM (each 1.0%).

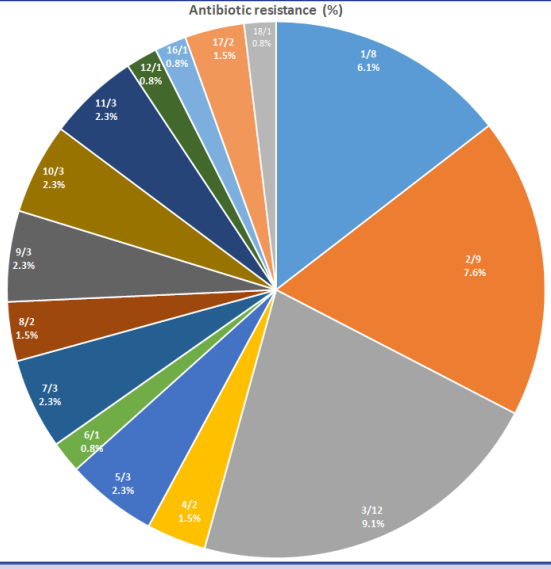

Figure 1. Antibiotic resistance rates (%) of 54 strains isolated from lakerda samples determined to be resistant to at least one antibiotic. Numerator (e.g., 1, 2, 3, etc.): Indicates the number of antibiotics to which the bacteria are resistant. Denominator (e.g., 8, 9, 12, etc.): Represents the number of bacterial isolates showing resistance to that specific number of antibiotics. For example, 1/8 means that 8 bacterial isolates were resistant to one antibiotic.

Among the isolated bacteria, 14 bacterial isolates with high similarity rates (≥99.1 to 100%) with gene bank records and definitive species identification were used in the study (Table 3).

Table 3. Bacterial isolate identification results.

Bacterial isolate	Accession numbers and Sequence result (similarity with 16S rRNA)
Pseudomonas fluorescens	AB204715.1 (99.3%)
Staphylococcus pasteuri	MF429378.1 (99.6%)
Staphylococcus haemolyticus	MT539735.1 (99.1%)
Staphylococcus equorum	MN758799.1 (99.1%)
Carnobacterium maltaromaticum isolate No. 1	MT631976.1 (99.9%)
Carnobacterium maltaromaticum isolate No. 2	MT631976.1 (99.9%)
Carnobacterium maltaromaticum isolate No. 3	MH119758.1 (100%)
Carnobacterium maltaromaticum isolate No. 4	MH119758.1 (100%)
Carnobacterium maltaromaticum isolate No. 5	MH119758.1 (100%)
Carnobacterium mobile	LT223645.1 (99.7%)
Vibrio hibernica isolate No. 1	MN796086.1 (99.4%) and NR_180997.1 (99.4%)
Vibrio hibernica isolate No. 2	MN796086.1 (99.4%) and NR_180997.1 (99.4%)
Vibrio rumoiensis isolate No. 1	KC534429.1 (99.8%)
Vibrio rumoiensis isolate No. 2	KC534429.1 (99.7%)

In this study, among the bacteria showing phenotypically multi antibiotic resistance in lakerda samples were *Pseudomonas fluorescens*, *Staphylococcus haemolyticus, Staphylococcus equorum*, *Carnobacterium maltaromaticum*, *Carnobacterium mobile* and *Vibrio hibernica* isolated (Table 4). In addition, antibiotic resistance genes in two more bacteria (*Vibrio rumoiensis* and *Staphylococcus pasteuri*) that did not have multiple antibiotic resistance was investigated. *V. rumoiensis* was chosen because it is a strong catalase producer. Detecting the presence of *V. rumoiensis* in Lakerda samples is important as it indicates that H_2O_2 may have been used in fillet bleaching. The presence of *S. pasture* in high-salt fermented various seafood dishes was reported, but it was not isolated in lakerda products before.

P. fluorescens is a regular contaminant of ready-to-eat foods and has been previously isolated from milk, dairy products, fish, chicken, beef, and vegetables (Kumar et al., 2019). In this study, it was determined that *P. fluorescens* isolate was resistant to 13 different antibiotics and carried genes associated with Extended Spectrum β-lactamases (*blaTEM*, *blaZ*), plasmid-mediated quinolone (*qnrB*, *qnrS*) and lincosamides (*msrA*) groups. The resistance of *Pseudomonas* spp. to β-lactam antibiotics is mainly attributed to Extended Spectrum β-lactamases (ESBLs) such as penicillin (1st, 2nd, and 3rd generations) and cephalosporins (cefotaxime, etc.) (Algammal et al., 2020). It was found that *P. fluorescens* isolated from lakerda carried resistance genes associat-

Table 4. Phenotypic and genotypic antibiotic resistance profiles of bacteria detected in lakerda samples.

Code	Bacteria	N*	Phenotype	Genotype
A	*Pseudomonas fluorescens*	1	OX, NA, VA, E, DA, C, KF, CRO, TE, CTX, AMC, SXT, AMP	*blaTEM, qnrB, qnrS, blaZ, msrA*
B	*Staphylococcus pasteuri*	1	K, S, CN	*mecA*
B	*Staphylococcus haemolyticus*	1	K, S, OX, CIP, NA, VA, E, DA, DO, IPM, C, KF, CRO, TE, CTX, AMC, SXT	*blaTEM, tetK, dfrD, blaZ,msrA, msrB, mecA*
A	*Staphylococcus equorum*	1	K, S, NA	*mecA*
C	*Carnobacterium maltaromaticum*	2	K, S, OX, CN, CIP, KF, CRO, NA, DA, CTX, AMP	*BlaTEM, mecA*
D	*Carnobacterium maltaromaticum*	3	K, S, OX, CN, NA, E, DA, KF, CRO, CTX, AMP	*blaTEM, qnrA, qnrB, qnrS, strA-strB, aphAI-IAB, mecA*
E	*Carnobacterium mobile*	1	K, S, OX, CIP, NA, VA, E, DO, DA, IPM, C, KF, CRO, TE, CTX, AMC, SXT, AMP	*blaTEM, blaZ, msrA, dfrD, mecA*
F	*Vibrio hibernica*	2	OX, KF, VA	*blaTEM, blaZ, mecA, VanA*
F	*Vibrio rumoiensis*	2	K, S, VA	-

*N: number of bacterial isolates
A, B and E: Fishermen samples
C, D and F: Fish market samples
AMC:AMOXYCILLIN / CLAVULANIC ACID 30 µG, AMP: AMPICILIN (AMP) (10 µg), E:ERYTHROMYCIN 15 µG, DA:CLINDAMYCIN 10 µG, VA:VANCOMYCIN 30 µG, OX:OXACILIN 5 µG, TE:TETRACYCLINE 30 µG, DO:DOXYCYCLINE HYDROCHLORIDE 30 µG, C:CHLORAMPHENICOL 30 µG, S:STREPTOMYCIN 10 µG, CN: GENTAMICIN 10 µG, K:KANAMYCIN 30 µG, NA:NALIDIXIC ACID 30 µG, CIP: CIPROFLOXACIN 5 µG, IMP: IMIPENEM 10 µG, SXT: SULPHAMETHOX. / TRIMETHOPRIM25 µG, CTX: CEFOTAXIME 30 µG, KF: CEPHALOTHIN 30 µG, CRO: CEFTRIAXONE 30 µG

ed with ESBLs (*blaTEM, blaZ*), plasmid-mediated quinolone (*qnrB*, *qnrS*), lincosamides (*msrA*) groups and methicillin/oxacillin resistance (*mecA*). Previous studies reported that *P. fluorescens* carried *blaTEM*gene in airborne isolates (Wang et al., 2022) and *qnrS* gene isolated from freshwater fish farms (Sherif et al., 2021). However, no study was found regarding *blaZ* and *msrA* genes in *P. fluorescens*.

A total of 222 isolates of *Pseudomonas* sp. were isolated in samples taken from a salmon processing company in Norway from surfaces (drain slaughter and drain filleting department) with or without food (fillet, skin, and gills, etc.) contact and 86% of them showed multi-antibiotic resistance (Thomassen et al., 2022). While these isolates are not phenotypically resistant to amikacin or tobramycin, it has been reported that the isolates are resistant to ampicillin, amoxicillin, oxolinic acid, florfenicol, cephalosporins, cefotaxime, ceftriaxone, and ciprofloxacin and genotypically carry the *adeF*, *soxR,* and *AbaQ* genes. Studies on antibiotic resistance of *P. fluorescens* isolated from salty food products are limited (Rodrigues et al., 2003). Rodrigues et al. (2003) reported that *Staphylococcus* species from gram-positive bacteria and *P. fluorescens* from gram-negative bacteria isolated from salted cod produced by different methods were intense. It was reported that *Pseudomonas* sp. isolated from sea bass (*Dicentrarchus labrax*) and shrimp (*Philocheras trispinosus*) were resistant to ampicillin, oxytetracycline, amoxicillin-clavulanic acid, trimethoprim/sulfamethoxazole, florfenicol, sulfamethoxazole and erythromycin (Güngör et al., 2021).

Bacteria in the *Staphylococcus* genus are pathogenic in mammals and many other living species. In this study, among three different *Staphylococcus* species, *S. haemolyticus* can cause opportunistic infections in immunosuppressed patients, especially hospitalized patients and those with medical implants. It is one of the coagulase-negative staphylococci that live commensal in the skin (Eltwisy et al., 2020).

It was found that *S. haemolyticus* isolated from lakerda was resistant to 17 different antibiotics and carried genes associated with ESBLs (*blaTEM, blaZ*), lincosamides (*msrA, msrB*), tetracycline (*tetK*), folate pathway inhibitors (*dfrD*) and methicillin/oxacillin resistance (*mecA*). Similarly, Regecová et al. (2014) reported that *S. haemolyticus* isolated from frozen Atlantic herring meat resisted erythromycin, oxacillin, and ampicillin antibiotics. *S. haemolyticus* isolated from fish meat showed resistance to ampicillin, oxacillin, tetracycline, and gentamicin (Hammad et al., 2012). Chajęcka-Wierzchowska et al. (2023) reported that *S. heamolyticus* isolated from fish tartar from ready-to-eat foods they bought from bars and restaurants were phenotypically resistant to gentamicin, clindamycin, erythromycin, cefoxitin, fusidic acid, norfloxacin, penicillin, tetracycline, and quinupristin/dalfopristin. They genotypically carried *aac(6)-Ie-aph(2")-Ia*, *blaZ*, *erm*A, *erm*B, *mec*A, *msr*(A/B), and *tet*K genes (Chajęcka-Wierzchowska et al., 2023).

This study determined that while *S. pasteuri* isolated from lakerda samples prepared by fishermen was resistant to K, S, and CN antibiotics, it carried the methicillin/oxacillin resistance gene *mecA*. *S. pasteuri* species isolated from jeotgal, a Korean food and a high-salt fermented dish [20–30% (w/w) salt] that can be made with a variety of seafood (fish, shrimp, oysters, scallops and caviar), was found to be resistant to linezolid, penicillin, and trimethoprim antibiotics and carried the *dfrA* gene associated with trimethoprim resistance (Lee & Jeong, 2015). Similarly, *S. pasteu-*

ri isolated from ready-to-eat foods (e.g., burgers, cheeses, juices, sushi, salads, sandwiches, meat, and fish tartare) in bars and restaurants were found to be resistant to gentamicin, clindamycin, erythromycin, cefoxitin, fusidic acid, penicillin, quinupristin/dalfopristin and rifampicin and carried *bla*Z, *erm*A, *erm*B, *mec*A, *msr*(A/B), *tet*K, and *tet*M genes (Chajęcka-Wierzchowska et al., 2023).

S. equorum is one of the coagulase-negative staphylococci isolated from fermented foods, bacterial surface-ripened cheeses, cattle, goats, horses, and sheep (Becker et al., 2014). No staphylococcal food poisoning via fermented foods has been associated with S. *equorum*, and no evidence of its pathogenicity has been reported. In this study, *S. equorum* isolated from lakerda samples showed resistance to K, S, and NA antibiotics carrying the *mecA* gene. *S. equorum* isolated from jeotgal are resistant to penicillin G, erythromycin, trimethoprim, lincomycin, and chloramphenicol; it was reported that they carry *lnuA* and *pbp* resistance genes (Jeong et al., 2014).

Carnobacterium is a lactic acid bacteria found in the natural environment and foods, and its positive and negative effects are still the subject of research (Leisner et al., 2007). It was previously isolated from aquatic products such as salted lumpfish, cold-smoked salmon, gravad rainbow trout, brine shrimp, seafood salad, cooked modified atmosphere packaging shrimp, and modified atmosphere packaging rough head grenadier (Françoise, 2010). *Carnobacterium* can be toxic to sensitive individuals because it can produce tyramine in food (Leisner et al., 2007). For this reason, it is crucial for public health to monitor *Carnobacterium* species in food and to investigate the antibiotic resistance of strains.

This study isolated three different *Carnobacterium* isolates with multiple antibiotic resistance from lakerda samples. One of the *Carnobacterium maltaromaticum* isolated from two other fish markets was found to be resistant to 11 different antibiotics. In addition, it was observed that one carried the *blaTEM* and *mecA* resistance genes, while the other had the *blaTEM*, *qnrA*, *qnrB*, *qnrS, strA-strB*, *aphAI-IAB,* and *mecA* resistance genes. In lakerda samples taken from fish markets, *Carnobacterium mobile* species were isolated and showed resistance to 18 antibiotics. It was found to carry *blaTEM*, *blaZ, msrA, dfrD, and mecA* resistance genes. Although *Carnobacterium* was previously isolated from 20-30% salty Geotgal product, its species could not be defined precisely, and antibiotic resistance information was not reported (Guan et al., 2011). A previous study reported that *Carnobacterium*isolated from food carried the *tetM*and *tetS* genes associated with tetracycline resistance (Li & Wang, 2010).

Vibrio species are among the bacterial species frequently isolated in aquatic ecosystems and food samples (Zeidler et al., 2024).

In this study, two different *Vibrio* species, *Vibrio rumoiensis* and *Vibrio hibernica* were isolated from lakerda samples taken from fish markets. Yumoto et al. (1999) isolated *V. rumoiensis* as a potent catalase producer from the drainage pond of a fish processing plant using H_2O_2 as a bleaching and microbial agent. The presence of this species in the lakerda samples produced in the facility suggests that H_2O_2 may have been used in the fillet bleaching. In addition, histamine-producing *V. rumoiensis* was isolated from the Chinese salted fish pickled overnight product (Tao et al., 2022).

In this study, *V. rumoiensis* isolated from lakerda obtained from the fish market showed resistance to K, S, and VA antibiotics. All of the *Vibrio* species isolated from water and sediment samples near the shrimp farm were resistant to at least one antibiotic, and the presence of *V. rumoiensis* was reported among the sediment isolates (dos Santos Rocha et al., 2016). It was reported that the marine sediment isolate *V. rumoiensis* is resistant to ampicillin, oxytetracycline, and penicillin (Rocha, 2011).

Woods et al. (2020) propose that *V. hibernica* is a member of the rumoiensis clade. It is an essential feature of an industrial food processing bacterium, and it was isolated in fermented foods and Greek table olives (Woods et al., 2020; Mougiou et al., 2023). This study observed that *V. hibernica* isolated from lakerda obtained from the fish market was resistant to OX, KF and VA antibiotics and carried *blaTEM*, *blaZ, mecA,* and *VanA* resistance genes. However, to our knowledge, the resistance genes we detected for the lakerda isolate *V. hibernica* have yet to be reported in this bacterial species before.

CONCLUSION

In this study, eight different bacterial isolates showing multiple antibiotic resistance were identified in prepared lakerda samples. The detected bacteria include *Pseudomonas fluorescens, Staphylococcus haemolyticus, Staphylococcus pasteuri, Staphylococcus equorum, Carnobacterium maltaromaticum, Carnobacterium mobile, Vibrio hibernica*, and *Vibrio rumoiensis*. Among the identified resistance genes, blaTEM and mecA were the most common and were found in almost all isolates, followed by blaZ and qnrB. The widespread presence of these genes highlights the significant risk posed by beta-lactam and methicillin/oxacillin resistance in bacteria associated with salted seafood. To minimize these risks, strict hygiene protocols should be implemented throughout the lakerda production and storage processes, especially in traditional preparation methods. Public health officials need to ensure hygiene conditions in the preparation of lakerda, determine the ways of transmission, and take precautions to raise awareness among producers and consumers.

Conflict of interest: There is no conflict of interest.

Ethics Committee Approval: The authors declare that this study did not include any experiments with human or animal subjects.

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