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The Effects of Antimicrobial Residues on Microbiological Content and the Antibiotic Resistance in Frozen Fish

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ABSTRACT

As fish are perishable foods, their storage conditions require appropriate sanitary and temperature regimes. The producers commonly use various antibiotics to stop fish's microbiological and biochemical processes. The current research aimed to examine antibacterial residues in frozen fish (Argentina, flounder, lackerda, mackerel, capelin, salka, saithe, herring, dorado, and pink salmon) to find their influence on the quantitative content of microorganisms and to determine the sensitivity of isolated psychrotrophic bacteria to antibiotics. A total of 75 samples were collected from the fillets of frozen fish species. These fish were imported from Norway (16 samples), Vietnam (24 samples), Russian Federation (8 samples), China (14 samples), New Zealand (2 samples), Italy (2 samples), United States (4 samples), and United Kingdom (5 samples). The obtained results revealed that aminoglycosides (Gentamicin, Kanamycin, Spectinomycin, Dihydrostreptomycin, Paromomycin, and Apramycin) were in 45.6 ± 1.4% of frozen fish. The findings indicated the presence of some antibacterial residues (Nalidixic acid, antibiotics: Apramycin, Kanamycin, Tiamulin, and Nafcillin) in frozen fish, the definition of which has not been specified in the EU Regulation. This gives grounds to prohibit the use or develop standards for the maximum permissible concentration of these antibacterial substances in fish. The most common psychrotrophic bacteria isolated from frozen fish without antibacterial residues were highly sensitive to antibiotics, including Penicillin, Tetracycline groups, and Aminoglycosides. Therefore, it can be concluded that the residual levels of various biocides found in fish are a source for the expression of multi-resistance genes, which can be transmitted to consumers in the food chain.

Keywords: Antibacterial residues, Antibiotic resistances, Frozen fish, Multi-resistance genes, Psychrotrophic microorganisms

INTRODUCTION

Fish and seafood are a source of easily digestible protein and contain fats, a valuable source of energy, as well as macro- and micronutrients (Al-Jasser and Al-Jasass, 2014; Nirmal et al., 2022). Fish is a nutrient medium for the development of microorganisms of all kinds (EC, 2002; Nirmal et al., 2022). They are classified as perishable foods, so the conditions and terms of their storage require appropriate sanitary, hygienic, and temperature regimes (Feng et al., 2017; Farag et al., 2021).

Fish and imported fish products enter the Ukrainian market in a frozen state. They are controlled according to criteria set for microbiological safety (presence of *Salmonella* spp., *Listeria monocytogenes*) and hygiene of the technological process (bacteria of the coliform group, mesophilic aerobic and facultative anaerobic microorganisms, *Staphylococcus aureus* (DSTU, 2007). Food stored in the refrigerator is dominated by psychrotrophic microflora (Grynevych et al., 2018; Zhang et al., 2019). Its development is related to product organoleptic and chemical defects (Ercolini et al., 2009; Moschonas et al., 2011; Malimon et al., 2018). Chilled and frozen fish are commonly contaminated with psychrotrophic microorganisms, such as *Pseudomonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Moraxella* spp., *Shewanella* spp., and *Aeromonas* spp., which can perish the fish (Franzetti and Scarpellini, 2007; Popelka et al., 2016) and cause food poisoning. Accordingly, producers widely use various antibacterials to stop microbiological and biochemical processes in the fish chain from catch to consumer (Akinbowale et al., 2007; Bayer et al., 2017). Antibacterial drugs are used to feed fish in aquaculture and to prevent and treat diseases (Samanidou and

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Evaggelopoulou, 2007; Rico et al., 2012; Grynevych et al., 2018; Zhang et al., 2019; Nasr-Eldahan et al., 2021). Researchers have investigated the antibacterial residues of different types of fish in Ukraine (Grynevych et al., 2018), China (Chen, 2014; Maan et al., 2021), Croatia (Kolda et al., 2020), and Australia (Al-Jasser and Al-Jasass, 2014). It is believed that excessive amounts of antibiotics lead to antibiotic-resistant bacteria forming in the aquatic environment, which can cause fish diseases (Grigorakis and Rigos, 2011; Su et al., 2011; Miller and Harbottle, 2018). Consequently, there is a possibility of resistance transmission to microorganisms that cause human food infections.

Thus, it is of utmost significance to study microbiological indicators, particularly psychrotrophic bacteria's content and residual amounts of antibacterial substances in frozen fish imported to Ukraine. Such studies can improve preventive measures to monitor and control residual levels of antibacterial drugs in aquatic products entering the fish market. With this in mind, the current research was an attempt to study antibacterial residues in frozen fish, their influence on the quantitative content of microorganisms, and to determine the sensitivity of isolated psychrotrophic bacteria to antibiotics.

MATERIALS AND METHODS

Sampling

A total of 75 samples were collected from the fillets of frozen fish species (Argentina, flounder, lackerda, mackerel, capelin, salka, saithe, herring, dorado, and pink salmon). The frozen fish were purchased from the trade network in Lviv and Ternopil, Ukraine. These fish were imported from Norway (16 samples), Vietnam (24 samples), the Russian Federation (8 samples), China (14 samples), New Zealand (2 samples), Italy (2 samples), the United States (4 samples), and the United Kingdom (5 samples). All samples of frozen fish had an acceptable shelf life of 6-9 months and were stored at a temperature of -18°C.

Microbiological study of fish

In this phase of the study, 48 cultures (18 *Pseudomonas* spp., 15 *Acinetobacter* spp., and 15 *Alcaligenes* spp.) from fish samples in the absence of antibacterial residues and 80 cultures (30 *Pseudomonas* spp., 25 *Acinetobacter* spp., and 25 *Alcaligenes* spp.) in the presence of antibacterial residues were studied.

The presence of antibacterial residues was determined by the chromatographic method (Waters mass spectrometer, TQD ACQITY system, USA). The antibiotics included Sulfaguanidine, Sulfacetamide, Sulfapyridine, Sulfadiazine, Sulfamethoxazole, Sulfathiazole, Sulfamerazine, Sulfamethizol, Sulfabenzamide, Sulfamethazine, Sulfinoxolin, Sulfadoxin, Sulfadimethoxine, Penicillin G, Cephalexin, Ampicillin, Penicillin V, Amoxicillin, Trimethoprim, Nafcillin, Oxacillin, Josamycin, Spiramycin, Nalidixic Acid, Flumequine, Oxalic Acid, Norfloxacin, Ciprofloxacin, Spectinomycin, Danofloxacin, Enrofloxacin, Marbofloxacin, Sarafloxacin, Difloxacin, Lincomycin, Gentamicin, Doxycycline, Chlortetracycline, Tetracycline, Ostetracycline, Kanamycin, Apramycin, Streptomycin, Dihydrostreptomycin, Paromomycin, Sulfamoxol, Sulfaphenazole, Sulfamethoxypyridazine, Sulfamonomethoxine, and Tiamulin (Di Corcia and Nazzari, 2002).

The number of mesophilic microorganisms was determined at a temperature of 30ºC incubation for 72 hours of Nutrient Agar (Pharmactive, Ukraine). The number of psychrotrophic microorganisms was calculated at a temperature of 6.5ºC incubation for 10 days on Nutrient Agar (Pharmactive, Ukraine). The NEFERM test 24 was used to identify isolated cultures of psychrotrophic microorganisms (Lachema, Czech Republic). The bacteria species were isolated according to the Bergey's Manual of Systematic Bacteriology (Vos et al., 2011). The psychrotrophic bacteria *Acinetobacter* spp., *Pseudomonas* spp., *Enterobacter* spp., *Alcaligenes* spp., and *Aeromonas* spp were identified. The sensitivity of the isolated bacteria to 14 antibiotics was tested by the classical disk-diffusion method Kirby-Bauer (NCCLS, 2003). The classical antibacterial disks used in this experiment consisted of Nalidixic acid, Tiamulin, Apromycin, Kanamycin, Sulfaphenazole, Gentamicin, Penicillin V, Difloxacin, Dihydrostreptomycin, Amoxicillin, Naphcillin, Spectinomycin, Tetracycline, Streptomycin, Paromomycin (Pharmactive, Ukraine). All procedures of this experiment were repeated three times.

Statistical analysis

The results were expressed as mean value and standard deviation (SD) of three measurements were calculated. Statistical processing was performed by analysis of variance (ANOVA) using SAS (Version 9.2). P value less than 0.05 was considered statistically significant.

RESULTS

In previous research, 10% of frozen fish samples imported into Ukraine contain residues of antibacterial substances. Figure 1 shows the chemical composition of the available residual antibacterial amounts in fish fillets. As can be seen in Figure 1, the antibacterial residues of Nalidixic acid was most often detected in frozen fish imported to Ukraine in 17.8 ±

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0.3% of samples. Apramycin and Kanamycin from Aminoglycosides antibiotics were detected in 16.3 ± 0.3% and 15.1 ± 0.3% of cases, respectively. The sulfonamide drug Sulfaphenazole was detected in 10% of the samples. In the investigated fish samples, Gentamicin and Tiamulin antibiotics were detected in almost the same amount of 6.9 ± 0.2 and 6.5 ± 0.2%, respectively. Such aminoglycoside antibiotics as Dihydrostreptomycin and Streptomycin were in a smaller number of fish samples (4.1 ± 0.2 and 1.6 ± 0.2%, respectively).

Among antibiotics of the Penicillin series, Penicillin V was most often detected in fish in 5.0 ± 0.2% of samples, and Amoxicillin and Nafcillin contained an average of 3.3 ± 0.1% of fish samples. Tetracycline and Paromomycin were detected in 1.7 ± 0.2% and 1.3 ± 0.1% of frozen fish samples, respectively.

Table 1 shows the permissible level of antibacterial residuals based on EU Regulation No. 37/2010. According to Table 1, Nalidixic acid, Apramycin, Kanamycin, Tiamulin, and Nafcilin antibacterials found in frozen fish were not based on regulations set by European legislation (EU Regulation No. 37/2010). In addition, the level of Gentamicin, Difloxacin, and Paromomycin found in the fish almost reached the maximum permissible amount allowed for these antibiotics. Amoxicillin and Penicillin V in the fish were twice lower than the maximum permissible amount according to EU Regulation 37/2010. At the same time, it was found that Tetracycline and Spectinomycin were above the maximum permissible amount of antibiotics in fish by 10% by 12.4%, respectively.

In the next phase of the study, microbiological indicators of frozen fish were determined based on antibacterial residuals. Various chemical preservatives influence the quantitative content of microorganisms in raw materials and food products. Figure 2 shows the number of microorganisms (mesophilic and psychrotrophic microflora) in frozen fish based on the detected antibacterial residues.

The findings revealed that the number of microorganisms in fish containing antibacterial residues of various pharmacological groups was 1.3-1.6 times lower than that of microflora in fish without antibiotics (Figure 2). Notably, in fish meat containing Tetracycline and Fluoroquinolone, the number of mesophilic microorganisms was 51.7 and 43.5 times ($p < 0.05$) less than in fish without antibiotics, respectively. This is probably due to the significant antibacterial effect of these antibiotics on mesophilic microflora. Regarding number of mesophilic microorganisms, all frozen fish samples met the requirements of the microbiological standard up to 5×10^4 CFU/g to SSU 4868: 2007 Frozen fish (DSTU, 2007).

In addition, it was found that in frozen fish without antibiotic residuals, the number of psychrotrophic microflora was 2.1 times greater than the number of mesophilic bacteria ($p < 0.05$), and in fish with antibiotic residuals, it was 1.3-1.7 times greater than the mesophilic bacteria ($p < 0.05$). For this reason, the microbiological control of frozen fish by psychrotrophic microflora content can determine the hygiene of the technological process involved in fish production and storage.

Regardless of the antibiotics in frozen fish, the dominant microflora is psychrotrophic microorganisms. Therefore, psychrotrophic microorganisms isolated from frozen fish were identified. It was found that the psychrotrophic microflora of frozen fish consists of *Acinetobacter* spp for 35-40%, *Pseudomonas* spp. for up to 30%, *Enterobacter* genus for 7-10%, and up to 20% for *Alcaligenes, Aeromonas*, cocci bacteria, and fungal microflora.

The current research was also conducted to determine the resistance of psychrotrophic microorganisms to antibiotics. The results are shown in Figures 3-7. Figure 3 illustrates the resistance of bacteria to antibiotics isolated from frozen fish without antibacterial residues. It can be inferred that in fish with no antibacterial residues, the isolated psychrotrophic microflora was mostly sensitive to antibiotics. The level of antibiotic-resistant strains of *Pseudomonas* spp., *Acinetobacter* spp., and *Alcaligenes* spp. did not exceed 20%. An exception was the Penicillin antibiotic Amoxicillin, which did not affect *Pseudomonas* spp. cultures, due to the natural resistance of these bacteria to Penicillins. Therefore, it was found that the most common psychrotrophic microorganisms isolated from frozen fish free from antibacterial preparations are mainly sensitive to antibiotics.

Figure 4 shows the results of antibiotic resistance of psychrotrophic bacteria isolated from frozen fish containing Tetracycline. As can be seen, isolated psychrotrophic microorganisms from fish containing Tetracycline were more resistant to antibiotics, compared to microorganisms isolated from fish without antibiotics. In particular, the level of bacterial cultures resistant to Tetracycline was 86.6-93.4%. At the same time, Ciprofloxacin, Ceftriaxone, and Gentamicin showed a stable antimicrobial action against isolated bacteria of the genus *Pseudomonas*, *Acinetobacter*, and *Alcaligenes*; the level of resistant strains did not exceed 26.7%.

The cephalosporin antibiotic Ceftazidime and the nitrofuran drug Furamag showed high antimicrobial activity. Since resistant bacteria of the genera *Acinetobacter* were not detected to these drugs, and the resistance of strains of *Pseudomonas* spp. was from 6.7 to 13.3%. Therefore, it was established that in case of the presence of antibiotics of the Tetracycline group in frozen fish, microflora resistant to drugs of this pharmacological group is released from the fish.

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The results of antibiotic resistance of psychrotrophic microorganisms isolated from fish containing Penicillin group preparations are shown in Figure 5.It was set that bacteria of the genera *Acinetobacter* and *Alcaligenes* developed resistance to antibiotics of the Penicillin group, as the number of resistant strains was 86.7-93.3% (Figure 5). The content of antibiotics can explain this in the Penicillin group in frozen fish. At the same time, antibiotics of other pharmacological groups showed high activity against isolated bacteria. The results of the research on antibiotic resistance of psychrotrophic microorganisms isolated from fish containing Gentamicin are shown in Figure 6. It was found that resistance to some specific antibiotics in frozen fish of samples containing Gentamicin residual is formed in the isolated microflora. In particular, the antibiotic Gentamicin was detected in frozen fish, as a result of which the resistance level of isolated bacteria of the genera *Pseudomonas*, *Acinetobacter*, and *Alcaligenes* were 86.7% and 93.3%, respectively (Figure 6).

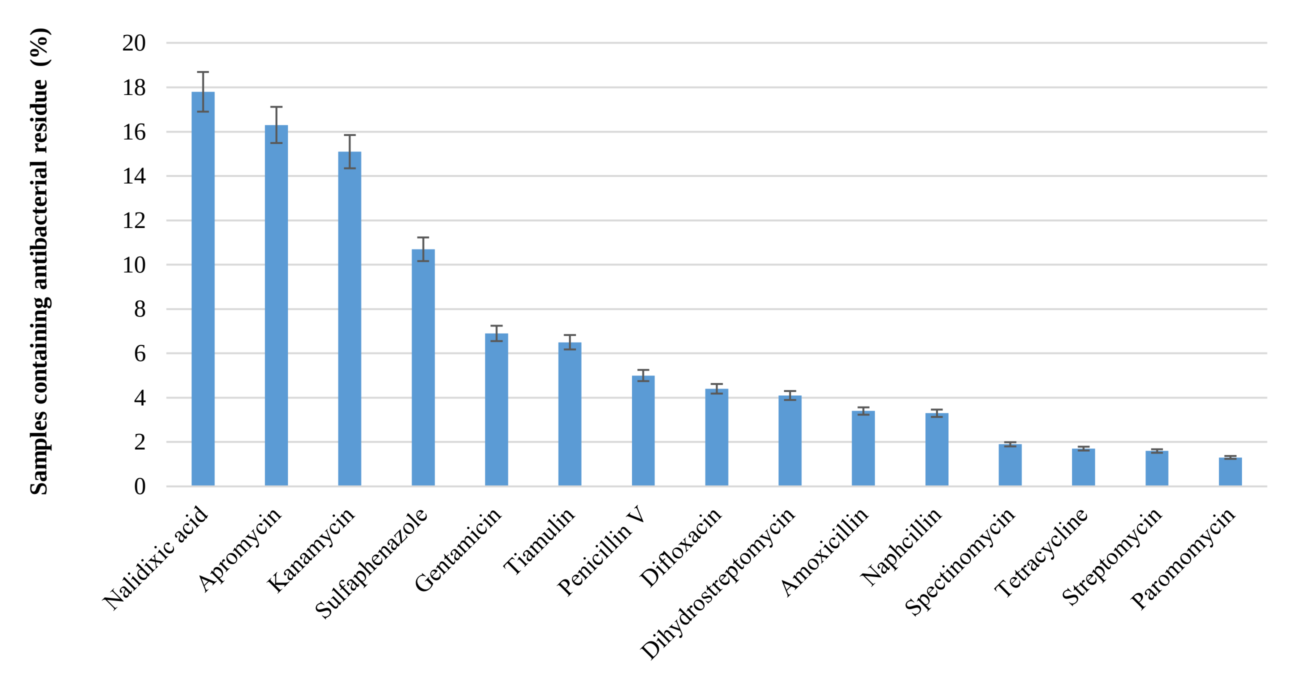

Figure 1. The percentage of fish samples containing antibacterial residue (n = 75, mean ± standard deviation)

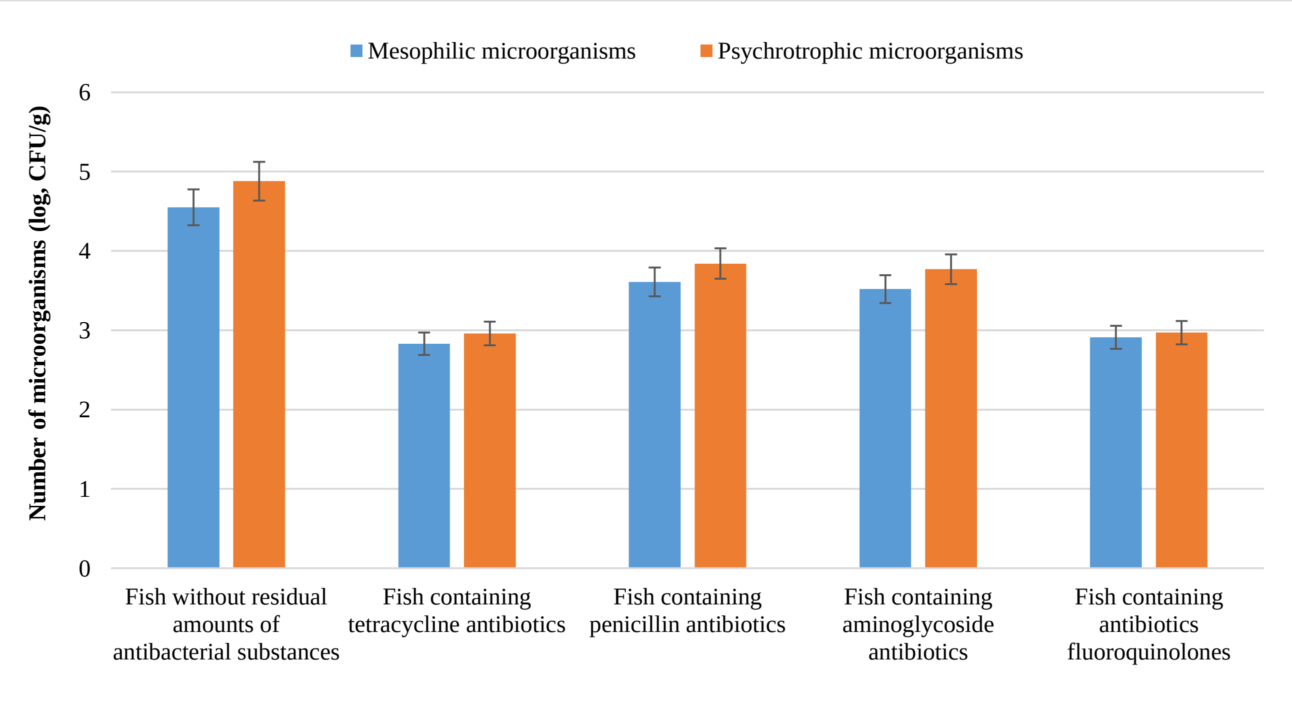

Figure 2. The number of mesophilic and psychrotrophic microflora in frozen fish fillets (n = 75) containing antibacterial residuals (mean ± standard deviation).

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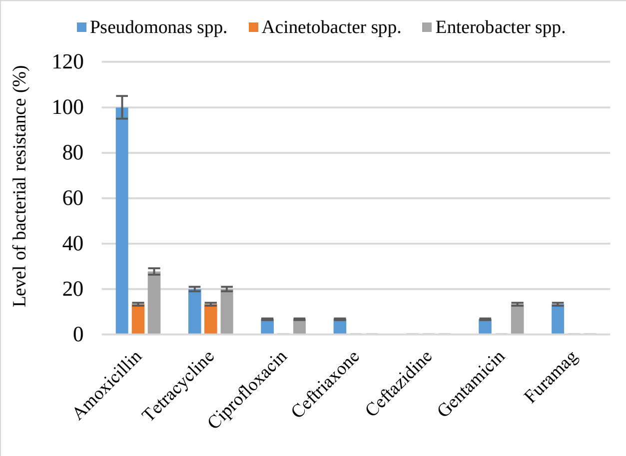

Figure 3. Antibiotic resistance of psychrotrophic microorganisms (mean ± standard deviation) isolated from fillets of frozen fish (n = 75) in the absence of antibacterial residues

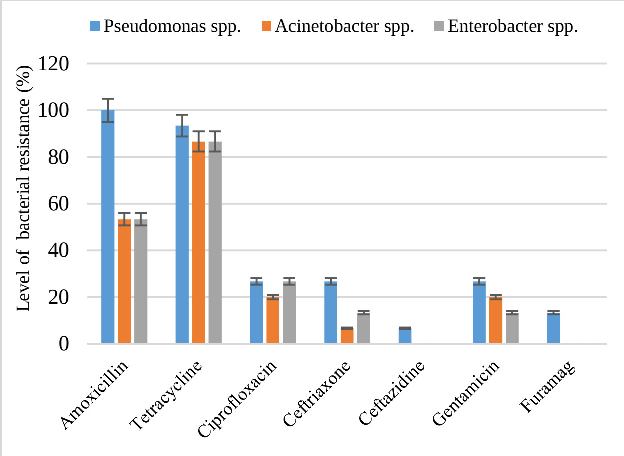

Figure 4. Antibiotic resistance of psychrotrophic microorganisms (mean ± standard deviation) isolated from frozen fish fillets (n = 75) with antibacterial residual of Tetracycline

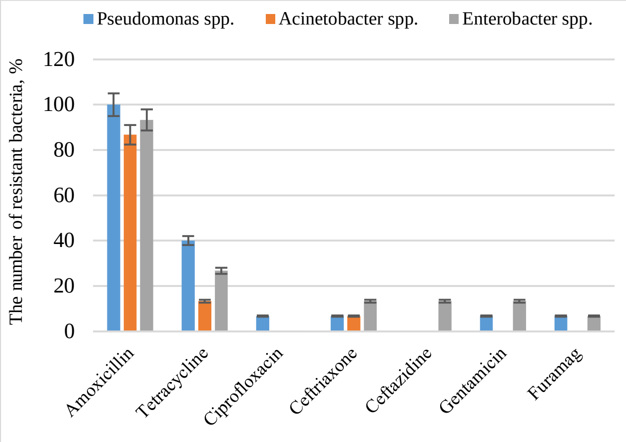

Figure 5. Antibiotic resistance (mean ± standard deviation) of psychrotrophic microorganisms (n = 75) isolated from frozen fish fillets containing antibacterial residual of the Penicillin group

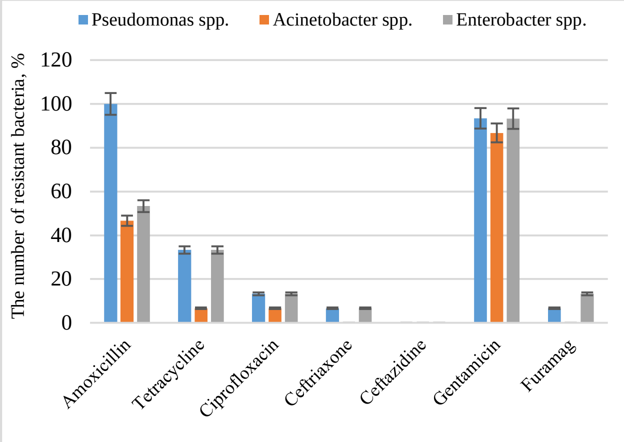

Figure 6. Antibiotic resistance (mean ± standard deviation) of psychrotrophic microorganisms (n = 75) isolated from frozen fish fillets containing antibacterial residual of Gentamicin

Table 1. Detection of antibacterial residuals in the fillets of frozen fish (n = 75)

Antimicrobial drugs	Concentration in fillet (mg.kg^{-1})	The maximum permissible level (mg.kg^{-1}) according to EU Regulation No. 37/2010
Nalidixic acid	90.5 ± 4.0	Not regulated in fish
Apramycin	881.3 ± 72.1	Not regulated in fish
Kanamycin	117.4 ± 50.3	Not regulated in fish
Sulfafenazole	75.2 ± 4.8	100
Gentamicin	94.9 ± 6.5	100
Tiamulin	78.9 ± 4.3	Not regulated in fish
Penicillin V	23.9 ± 2.0	50
Difloxacin	97.8 ± 4.3	100
Dihydrostreptomycin	361.5 ± 23.1	500
Amoxicillin	27.2 ± 2.2	50
Nafcillin	163.5 ± 10.6	Not regulated in fish, only for ruminants – 300
Spectinomycin	337.2 ± 18.2	300
Tetracycline	110.4 ± 6.5	100
Paromomycin	459.7 ± 28.1	500

DISCUSSION

The basic principles of food safety set out in the EU Regulation (EC, 2002) provide that food products are required to ensure traceability throughout the production and circulation chain. The obtained results of imported fish for residual

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amounts of antibacterial residues showed that the most commonly found antibiotics (45.6 ± 1.4 percentage of cases from the collected samples) are aminoglycosides (Apramycin, Kanamycin, Gentamicin, Spectinomycin, Paromomycin, Dihydrostreptomycin). The detection of almost 50% of residual amounts of antibiotics in this group is probably due to a wide range of antimicrobial action on Gram-negative and Gram-positive microorganisms, which are the causative agents of fish spoilage and human food poisoning. In addition, in almost 18% of cases, nalidixic acid was detected, which is probably due to its good activity against bacteria of the family *Enterobacteriaceae* (Casagrande Proietti et al., 2022). Other studies indicated the significant use of aminoglycosides and macrolides in animal husbandry, fish farming, and poultry farming, which can be detected through the residual analysis of these biocides in raw materials and products (Cabello, 2004; Su et al., 2011; Horiuk et al., 2019). The results are consistent with those of other researchers reported for foods, antibiotics, sulfonamides, and nitrofuran preparations (Akinbowale et al., 2007; Bayer et al., 2017).

In addition, it should be noted that the EU Regulation does not regulate residues of nalidixic acid, antibiotics such as apramycin, kanamycin, tiamulin and nafcillin in meat (EC, 2010). Exceeding the permissible content values was found for antibiotics such as Tetracycline by 10 mg.kg-1 and spectinomycin by 37.2 mg.kg^{-1} (EC, 2010). It is reported that the maximum allowable concentration of antibiotics in Tetracycline products has been exceeded (Miranda et al., 2003; Akinbowale et al., 2007; Su et al., 2011).

Thus, the results revealed that during the production of frozen fish, antibacterial substances are used, which is not provided by the EU Regulation (EC, 2010). This gives grounds to prohibit the use or development of standards for the maximum permissible concentration of these antibacterial substances in fish. In addition, the maximum allowable amount for antibiotics such as Tetracycline and Spectinomycin was exceeded. The present study also necessiates the need for careful control of frozen fish imported to Ukraine for the presence of residual amounts of antibacterial substances.

In the presence of residues of antibacterial substances in frozen fish, microbiological indicators did not exceed the standards (DSTU, 2007). The lowest microbial contamination of fish was detected in the presence of antimicrobial residues of Fluoroquinolone, Tetracycline groups, and Aminoglycosides. The obtained data are consistent with studies by Popelka et al. (2016), and Sheng and Wang (2021), which report higher significant contamination of frozen fish with psychrotrophic microflora, compared to mesophilic. Therefore, it can be considered that the microbiological characteristics of frozen fish in terms of the content of psychrotrophic microorganisms are more indicative and reliable for assessing compliance with the hygiene of production and refrigerated storage.

Various biocides are widely used in aquaculture to prevent and treat infectious diseases (Grynevych et al., 2018). The use of antibacterial substances in modern animal husbandry and aquaculture causes a global problem of antibiotic resistance of microorganisms (Kemper, 2008; Mulcahy, 2011; WHO, 2011; Horiuk et al., 2019). Studies on determining the sensitivity of the identified microflora to antibiotics have shown that in fish in which there are no antibacterial drugs, there are psychrotrophic bacteria, which are mainly sensitive to antibiotics. The resistance level of *Pseudomonas* spp., *Acinetobacter* spp., and *Alcaligenes* spp. did not exceed 20%. At the same time, in the presence of residual amounts of antibiotics of the Tetracycline group in frozen fish, microflora resistant to these antibiotics was isolated. A similar pattern was found in the presence of antibiotics in other groups. Thus, Gentamicin residues were detected in the meat of frozen fish and, as a result, isolated from psychrotrophic microorganisms showed resistance to Gentamicin in 86.7-93.3%t. Some studies also reported antibiotic resistance in microorganisms isolated from aquaculture (Miranda et al., 2003; Akinbowale et al., 2007; Su et al., 2011). In particular, resistant bacteria to antibiotics of the Penicillin group (ampicillin), Tetracycline group (Tetracycline and oxytetracycline), and florfenicol were isolated. The researchers believe that the presence of residues of antibacterial substances in raw materials or products causes antibiotic resistance in bacteria (Kemper, 2008; Mulcahy, 2011; Horiuk et al., 2019).

Therefore, the results of the current studies could support the findings of other studies indicating that the residual amounts of various biocides found in fish are a source of microorganisms with multi-resistance genes to antibiotics.

CONCLUSION

Aminoglycoside antibiotics (Gentamicin, Kanamycin, Spectinomycin, Dihydrostreptomycin, Paromomycin, and Apramycin) were most often detected in frozen fish in a total amount of 45.6 ± 1.4% of the tested samples. Antibacterial preparations (Nalidixic acid, Apramycin, Kanamycin, Tiamulin, and Nafcillin) were detected in frozen fish, which is not specified in the EU Regulation (No. 37/2010). Moreover, the amount of antibiotics, such as Tetracycline and Spectinomycin in fish exceeds the maximum permissible amount. In the absence of antibacterial preparations in frozen fish, the existing microflora is highly sensitive to most antibiotics. At the same time, microflora resistant to the identified antibiotics forms in frozen fish with the remains of antibacterial preparations. Therefore, to prevent the receipt of fish with antibacterial substances to consumers and the spread of antibiotic-resistant strains of bacteria through fish, it is necessary to introduce careful control over the safety of frozen fish. Future experiments can be conducted to determine antibiotic residues in frozen seafood (mussels, shrimps, rapans) exported to Ukraine.

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DECLARATIONS

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Authors' contribution

Mykola Kukhtyn, Zoya Malimon, Volodymyr Salata, Igor Rogalskyy, Bogdan Gutyj developed an experiment, analyzed data, and wrote the manuscript. Larysa Kladnytska, Khrystyna Kravcheniuk, and Yulia Horiuk helped with the manuscript writing setting and data analysis. All authors checked and approved the final version of the manuscript for publication in the present journal.

Competing interests

The authors declare that they have no conflict of interest.

Ethical considerations

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors.

REFERENCES

Akinbowale OL, Peng H, and Barton MD (2007). Diversity of tetracycline resistance genes in bacteria from aquaculture sources in Australia. Journal of Applied Microbiology, 103(5): 2016-2025. DOI: https://www.doi.org/10.1111/j.1365-2672.2007.03445.x

Al-Jasser MS and Al-Jasass FM (2014). Study the chemical, physical changes and microbial growth as quality measurement of fish. Annual Research & Review in Biology, 4(9): 1406-1420. DOI: https://www.doi.org/10.9734/ARRB/2014/7131

Bayer EV, Novozhitskaya YN, Shevchenko LV, and Mykhalska VM (2017). Monitoring of residues of veterinary preparations in food products. Ukrainian Journal of Ecology, 7(3): 251-257. DOI: https://www.doi.org/10.15421/2017_76

Cabello FC (2004). Antibiotics and aquaculture in Chile: implications for human and animal health. Revista medica de Chile, 132(8): 1001-1006. DOI: https://www.doi.org/10.4067/s0034-98872004000800014

Casagrande Proietti P, Musa L, Stefanetti V, Orsini M, Toppi V, Branciari R, and Franciosini MP (2022). mcr-1-Mediated Colistin Resistance and Genomic Characterization of Antimicrobial Resistance in ESBL-Producing Salmonella Infantis Strains from a Broiler Meat Production Chain in Italy. Antibiotics, 11(6): 728. DOI: https://www.doi.org/10.3390/antibiotics11060728

Chen H (2014). Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. Marine Pollution Bulletin, 3: 277-282. DOI: https://www.doi.org/10.1016/j.marpolbul.2014.10.053

Di Corcia A and Nazzari M (2002). Liquid chromatographic–mass spectrometric methods for analyzing antibiotic and antibacterial agents in animal food products. Journal of Chromatography A, 974: 53-89. DOI: https://www.doi.org/10.1016/S0021-9673(02)00905-6

EC (2002). Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002R0178-20140630&rid=1.

EC (2010). Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin (Text with EEA relevance). Available at: https://ec.europa.eu/health/sites/default/files/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf

Ercolini D, Russo F, Nasi A, Ferranti P, and Villani F (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. Applied and Environmental Microbiology, 75(7): 1990-2001. DOI: https://www.doi.org/10.1128/AEM.02762-08

Farag MA, Abib B, Tawfik S, Shafik N, and Khattab AR (2021). Caviar and fish roe substitutes: Current status of their nutritive value, bio-chemical diversity, authenticity and quality control methods with future perspectives. Trends in Food Science & Technology, 110: 405-417. DOI: https://www.doi.org/10.1016/j.tifs.2021.02.015

Feng X, Ng VK, Mikš-Krajnik M, and Yang H (2017). Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage. Food and Bioprocess Technology, 10(1): 89-102. DOI: https://www.doi.org/10.1007/s11947-016-1798-7

Franzetti L and Scarpellini M (2007). Characterization of Pseudomonas spp. isolated from foods. Annals of Microbiology, 57(1): 39-47. DOI: https://www.doi.org/10.1007/BF03175048

Grigorakis K and Rigos G (2011). Aquaculture effects on environmental and public welfare – the case of Mediterranean mariculture. Chemosphere, 85(6): 899-919. DOI: https://www.doi.org/10.1016/j.chemosphere.2011.07.015

Grynevych N, Sliusarenko A, Dyman T, Sliusarenko S, Gutyj B, Kukhtyn M, Hunchak V, and Kushnir V (2018). Etiology and histopathological alterations in some body organs of juvenile rainbow trout Oncorhynchus mykiss (Walbaum, 1792) at nitrite poisoning. Ukrainian Journal of Ecology, 8(1): 402-408. DOI: https://www.doi.org/10.15421/2018_228

Hassan MA, Shaltout FA, Maarouf AA, and El-Shafey WS (2015). Psychrotrophic bacteria in frozen fish with special reference to pseudomonas species. Benha Veterinary Medical Journal, 27(1): 78-83. Available at: https://bvmj.bu.edu.eg/issues/27-1/7.pdf

Horiuk Y, Kukhtyn M, Kovalenko V, Kornienko L, Horiuk V, and Liniichuk N (2019). Biofilm formation in bovine mastitis pathogens and the effect on them of antimicrobial drugs. Independent Journal of Management & Production, 10(7): 897-910. DOI: https://www.doi.org/10.14807/ijmp.v10i7.1012

Kemper N (2008). Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators, 8(1): 1-13. DOI: https://www.doi.org/10.1016/j.ecolind.2007.06.002

Kolda A, Mujakić I, Perić L, Vardić Smrzlić I, and Kapetanović D (2020). Microbiological quality assessment of water and fish from Karst Rivers of the Southeast Black Sea Basin (Croatia), and antimicrobial susceptibility of Aeromonas isolates. Current Microbiology, 77(9): 2322-2332. DOI: https://www.doi.org/10.1007/s00284-020-02081-5

Maan MK, Weng Z, Dai M, Liu Z, Hao H, Cheng G, and Huang L (2021). The Spectrum of Antimicrobial Activity of Cyadox against Pathogens Collected from Pigs, Chicken, and Fish in China. Antibiotics, 10(2): 153. DOI: https://www.doi.org/10.3390/antibiotics10020153

Malimon ZV, Kukhtyn MD, and Perkiy YB (2018). Contamination of frozen fish with mesophilic and psychrotrophic microorganisms depending on biochemical quality indices. Theoretical and Applied Veterinary Medicine, 6(3): 39-43. DOI: https://www.doi.org/10.32819/2018.63008

Miller RA and Harbottle H (2018). Antimicrobial drug resistance in fish pathogens. Microbiology Spectrum, 6(1): 1-20. DOI: https://www.doi.org/10.1128/microbiolspec.ARBA-0017-2017

Miranda CD, Kehrenberg C, Ulep C, Schwarz S, and Roberts MC (2003). Diversity of tetracycline resistance genes in bacteria from Chilean salmon farms. Antimicrobial Agents and Chemotherapy, 47(3): 883-888. DOI: https://www.doi.org/10.1128/AAC.47.3.883-888.2003

Moschonas G, Bolton DJ, McDowell DA, and Sheridan JJ (2011). Diversity of culturable psychrophilic and psychrotrophic anaerobic bacteria isolated from beef abattoirs and their environments. Applied and Environmental Microbiology, 77(13): 4280-4284. DOI: https://www.doi.org/10.1128/AEM.01778-10

Mulcahy DM (2011). Antibiotic use during the intracoelomic implantation of electronic tags into fish. Reviews in Fish Biology and Fisheries, 21(1): 83-96. DOI: https://www.doi.org/10.1007/s11160-010-9190-6

Myers ML and Durborow RM (2012). Aquacultural safety and health. Health and environment in aquaculture/in E. Carvalho (Chapter 15). pp. 385-400. Available at: https://www.semanticscholar.org/paper/Aquacultural-Safety-and-Health-Myers-Durborow/1afeb728b0409206dff88f88dedf041600bf3d3a

Nasr-Eldahan S, Nabil-Adam A, Shreadah MA, Maher AM, and El-Sayed Ali T (2021). A review article on nanotechnology in aquaculture sustainability as a novel tool in fish disease control. Aquaculture International, 29(4): 1459-1480. DOI: https://www.doi.org/10.1007/s10499-021-00677-7

National Committee for Clinical Laboratory Standards (2003). Approved standard: M2-A8. Performance standards for antimicrobial disk susceptibility tests, 8th edition. National Committee for Clinical Laboratory Standards, Wayne, Pa. Available at: https://clsi.org/media/1925/m02ed13_sample.pdf

Nirmal NP, Santivarangkna C, Benjakul S, and Maqsood S (2022). Fish protein hydrolysates as a health-promoting ingredient—recent update. Nutrition Reviews, 80(5): 1013-1026. DOI: https://www.doi.org/10.1093/nutrit/nuab065

Popelka P, Jevinová P, and Marcinčák S (2016). Microbiological and chemical quality of fresh and frozen whole trout and trout fillets. Potravinarstvo Slovak Journal of Food Sciences, 10(1): 431-436. DOI: https://www.doi.org/10.5219/599

Rico A, Satapornvanit K, Haque MM, Min J, Nguyen PT, Telfer TC, and van den Brink PJ (2012). Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: A critical review. Reviews in Aquaculture, 4(2): 75-93. DOI: https://www.doi.org/10.1111/j.1753-5131.2012.01062.x

Samanidou VF and Evaggelopoulou EN (2007). Analytical strategies to determine antibiotic residues in fish. Journal of Separation Science, 30(16): 2549-2569. DOI: https://www.doi.org/10.1002/jssc.200700252

Sheng L and Wang L (2021). The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. Comprehensive Reviews in Food Science and Food Safety, 20(1): 738-786. DOI: https://www.doi.org/10.1111/1541-4337.12671

Solomon O, Kingsley A, and Anosike S (2017). Effects of salts on preservation and metabolic activities of fish and meat microflora. Journal of Industrial Research and Technology, 6(1): 90-102. Available at: https://earthwormexpress.com/wp-content/uploads/2019/01/effects-of-salts-on-preservation-and-metabolic-activities-of-food-microflora.pdf

State standard of Ukraine DSTU (2007). State standard of Ukraine 4868:2007, Fish frozen. Specification. National Standard of Ukraine. Available at: https://ses-help.org.ua/dstu/ДСТУ%204868-2007%20риба%20заморожена.pdf

Su HC, Ying GG, Tao R, Zhang RQ, Fogarty LR, and Kolpin DW (2011). Occurrence of antibiotic resistance and characterization of resistance genes and integrons in *Enterobacteriaceae* isolated from integrated fish farms in south China. Journal of Environmental Monitoring, 13(11): 3229-3236. DOI: https://www.doi.org/10.1039/C1EM10634A

Topic Popovic N, Benussi Skukan A, Dzidara P, Coz-Rakovac R, Strunjak-Perovic I, Kozacinski L, Jadan M, and Brlek-Gorski D (2010). Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic coast of Croatia. Veterinarni Medicina, 55(5): 233-241. Available at: https://agris.fao.org/agris-search/search.do?recordID=CZ2010000551

Velu S, Bakar AF, Mahyudin NA, Saari N, and Zaman MZ (2013). Effect of modified atmosphere packaging on microbial flora changes in fishery products. International Food Research Journal, 20(1): 17-26. Available at: https://www.semanticscholar.org/paper/Effect-of-modified-atmosphere-packaging-on-flora-in-Velu Bakar/ce43e954196f671f589abcfc9afc90d575a48051

Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, and Whitman WB (2011). Bergey's Bergey’s manual of systematic bacteriology. Springer Science & Business Media, 3: 1450. Available at: https://link.springer.com/book/10.1007/978-0-387-68489-5#affiliations

World Healh Organization (WHO) (2011). Tackling antibiotic resistance from a food safety perspective in Europe. World Health Organization. Regional Office for Europe. Available at: https://apps.who.int/iris/bitstream/handle/10665/326398/9789289014212-eng.pdf

Zhang Y, Wei J, Yuan Y, and Yue T (2019). Diversity and characterization of spoilage-associated psychrotrophs in food in cold chain. International Journal of Food Microbiology, 290: 86-95. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2018.09.026

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