Vol 3 No 1 March 2022 Pages 1-9 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: http://journal.safebd.org/index.php/jafe

http://doi.org/10.47440/JAFE.2022.3101

Original Article

Effective Antibiotics Against Pathogenic Bacteria Isolated From Herring and Fesikh's Salted Fish Widely Consumed During the National Egyptian Day of Sham El-Nessim

Abd El-Fatah SI[1], Abdelmotilib NM[2], Ahmed MBM[1]*

[1]Food Toxicology and Contaminants Department, National Research Centre, 33 El Buhouth St., P.O. Box: 12622, Dokki, Cairo, Egypt.
[2]Department of Food Technology, City of Scientific Research and Technology Applications (SRTA- CITY), New Borg El-Arab City, Alexandria, Egypt.

Article History

Received: 03 December 2021

Revised: 27 February 2022

Accepted: 10 March 2022

Published online: 31 March 2022

***Corresponding Author**

Mohamed Bedair M. Ahmed, E-mail: m.bedair.nrc@gmail.com
md.bedair@nrc.sci.eg

Keywords

Antibiotic susceptibility; bacterial isolates; Herring and Fesikh fish

How to cite: Abd El-Fatah SI, Abdelmotilib NM, Ahmed MBM (2022). Effective antibiotics against pathogenic bacteria isolated from Herring and Fesikh's salted fish widely consumed during the national Egyptian day of Sham El-Nessim. J. Agric. Food Environ. 3(1): 1-9.

A B S T R A C T

Herring and Fesikh are the most popular traditionally smoked and salted fish in Egypt, especially on the day of Sham El-Nessim occasion. Consuming these traditionally manufactured products exposes consumers to some microbial infection. In this study, the microbial content of Herring and Fesikh samples collected from Egyptian markets located at Cairo and Alexandria cities has been examined. Also, the antibiotic-susceptibility pattern for the isolated pathogens was evaluated against the commonly used antibiotics. The results revealed that the mean values of total plate count, halophilic microorganisms, anaerobic spore formers, *E. coli*, *Staphylococcus aureus*, and total molds and yeasts were 5.2, 4.5, 1.4, 1.3, 4.3, and 4.3 log CFU/g of Herring samples, respectively, and 3.4, 2.5, 2.1, 2.6, 2.8 and 2.9 log CFU/g of Fesikh samples, respectively. For Herring samples, *E. coli* isolates showed multi-resistance against four cefoperazone, piperacillin, cefotaxime, and trimethoprim/sulphamethoxazole antibiotics, while *Staphylococcus aureus* isolates were only resistant to clindamycin. Concerning Fesikh samples, *Staphylococcus aureus* isolates had a multi-resistance pattern against seven antibiotics (clindamycin, azithromycin, cefoperazone, cefadroxil, piperacillin, amoxicillin, and cefotaxime). Noteworthy, the results of the antibiotic-susceptibility test revealed that antibiotic classes, which had significant effectiveness against the isolated bacteria, can be arranged as: aminoglycosides ≥ cephalosporines > tetracyclines.

Introduction

Sham El-Nessim day is an Egyptian traditional festival, which has been referred to as the spring festival from early ancient times. Four thousand and five hundred years ago, the ancient Egyptian used to celebrate by Sham El-Nessim occasion on the first Monday following the Coptic Easter festival. Sham El-Nessim is defined by Egyptian people as sniffing the breeze which means the spring weather begins. Egyptians often celebrate this day outdoors in the gardens and also have a habit to eat special food such as salted fish and smoked fish.

Salted and smoked fish, such as Fesikh and Herring, are more popular in numerous countries and counted as traditional products which are prepared by salting and smoking methods. Nevertheless, these fish are at the top of the list of foods linked to foodborne outbreaks (Yang *et al*., 2015) due to the poor processing techniques, treating conditions of un eviscerated fish, and the uncontrolled usage of antibiotics in fish farms increase the microbial load and resistance (Feldhusen, 2000). Individuals, who consume these fish, may be vulnerable to foodborne illness because they are consuming these fish either in raw or undercooked forms (Mizan *et al*., 2015). The severity of foodborne outbreaks can be ranged from mild gastroenteritis, such as abdominal pain and diarrhea, to severe life-threatening infections agents as kidney failure and even death. Most seafood-borne diseases are caused by viable bacterial pathogen origin and/or uptake of the biotoxins. Several types of bacterial species can cause food-borne diseases, after ingesting the contaminated food, like *Listeria*

monocytogenes, *Vibrio parahaemolyticus*, *Escherichia coli 0157:H7*, *Salmonella*, *Staphylococcus aurues*, *Clostridium perfringins*, *C. Botulinum* and *Shigella* (Al-Busaidi *et al*., 2016; Hosseini *et al*., 2004; Iwamoto *et al*., 2010). Salted and smoked fish could pose serious problems to public health, especially when pathogens that are resistant to antibiotics are present in these products (Dobiasova *et al*., 2014).

Antibiotics are essential drugs in the medical treatment of infectious diseases and foodborne illness, having several applications in several fields including veterinary, agriculture, and aquaculture. Broad-spectrum antibiotics are effective against multi groups of pathogenic bacteria, while narrow-spectrum antibiotics have a limited effect against specific types of bacteria. Tetracyclines, sulfonamides, and fluoroquinolones are mainly used in fish farming due to their active effect against Gram-negative and Gram-positive bacteria, however, these antibiotic residues can persist in fish tissues (Ahmed *et al*., 2020; Darwish *et al*., 2013; Samanidou & Evaggelopoulou, 2007).

The misuse of antibiotics in human and veterinary medicine and the overuse of disinfectants is increasing the problem of antibiotic resistance in bacteria (Rodriguez-Mozaz *et al*., 2015; Sharma *et al*., 2016). Several studies reported the resistance of some isolated pathogenic bacteria against multi classes and different categories of antibiotics such as sulfonamides, b-lactams, aminoglycosides, chloramphenicol, streptomyces, and penicillins (Kümmerer, 2009; Marti *et al*., 2014; Nguyen *et al*., 2014; Sharma, *et al*., 2016). The crisis of antibiotic resistance is responsible for hospital's burden through increasing the deaths and economic burden to the health care organization (Zhabiz *et al*., 2014). There is substantial evidence that antibiotic-resistant bacteria are spread in seafood and can cause a great threat to human health worldwide. So, the usage of antibiotics in aquaculture should be kept under veterinary supervision to minimize the risk of originating antibiotic resistance (Elbashir *et al*., 2018; Mizan, *et al*., 2015). Additionally, from time to time, isolated pathogens from food items should be tested for their susceptibility against the commercial antibiotics in the drug store to help physicians in the prescription of antibiotics to treat the foodborne illness.

Therefore, this study aimed to isolate the bacterial pathogens from the traditional Egyptian smoked (Herring) and salted fish (Fesikh) products; specifically in Sham El-Nessim occasion where poisoning cases are expected for some individuals from the spoiled fish. Also, determining the susceptibility of the isolated pathogenic bacteria against the commonly used antibiotics to be of help with physicians when prescribing antibiotics for the treatment of individuals exposed to pathogens from these traditional fish products.

Material and methods

Samples collection

Twenty samples of each of smoked fish (Herring; *Clupea harengus*) and salted fish (Fesikh; *Mugil cephalus*) were randomly collected, during Sham El-Nessim occasion of 2021, from twenty different local markets in Cairo and Alexandria cities (10 markets in each city), Egypt. Each sample was kept in a separate bag, transferred to the laboratory under aseptic conditions and stored in the refrigerator until analysis. Fig. (1) shows Fesikh and Herring samples.

Smoked Herring Fesikh

Fig. 1. Smoked Herring and Fesikh samples

Microbiological analysis of Herring and Fesikh samples

Twenty five grams were obtained from different tissues of each sample and homogenized with 225 mL of sterile peptone water (0.1%) and several serial dilutions have been made. Then, the appropriate dilution has been used in the microbiological analyses

Determination of total plate count (TPC) by using serial dilution and pour plate technique on nutrient agar and incubation was performed at 35±2 ^{o}C for 48h. Enumeration of *E. coli* by using *E. coli* broth media. Mannitol salt agar with egg yolk emulsion was used for the enumeration of *Staphylococcus aureus*. Total halophilic microorganisms count was determined using nutrient agar supplemented with 6% NaCl, and the plates were incubated at 35^{o}C for 48h. Isolation of mold and yeast count on potato dextrose agar and incubation at 25±2 ^{o}C for 5 days. Detection of anaerobic spore former bacteria by using RCM semi-solid agar tubes (Adesoji *et al*., 2019; Gassem, 2019). Detection of *Listeria monocytogenes* was done based on ISO 11290-1:2017 (E) (11290-1:2017, 2017). Detection of *Vibrio parahaemolyticus* was performed according to ISO 21872-1:2017-06 (21872-1:2017-06, 2017).

Purification and identification of isolated bacterial strains

Eighteen random single colonies from different media were isolated and purified by streaking repeatedly on the fresh plates of the corresponding media and incubated at 30^{o}C for 24hr. Bacterial isolates were identified using the morphologic characterization, Gram staining, and biochemical tests according to Gufe *et al*. (2019).

Antibiotic susceptibility testing assay

Antibiotics under test: Twelve commercial antibiotic discs were used for testing the antibiotic susceptibility for the isolated bacteria. These antibiotics belong to different antibiotic groups. The names and concentrations of the used antibiotics are as follows:

Amikacin 30 µg (AK-30), Gentamycin 10 µg (CN-10), Vancomycin 30 µg (VA-30), Doxycycline 30 µg (DO-30), Clindamycin 2 µg (DA-2), Azithromycin 15 µg (AZM-15), Cefoperazone 75 µg (CEP-75), Cefadroxil 30 µg (CFR-30), Piperacillin 100 µg (PRL-100), Amoxicillin/Clavuolanic acid 20/10 µg (AMC-30), Cefotaxime 30 µg (CTX-30) and finally Trimethoprim/Sulphmethoxazole 1.25/23.75 µg (SXT-25).

Disc diffusion assay: From the twenty four hours incubated isolates of each bacterial species, a loopful of the tested isolates was inoculated in a tube containing 5 ml of tryptic soy broth. The broth culture was incubated at 35^{o}C for two-six hours until it achieved the turbidity of 0.5 McFarland standard. The susceptibility to different commercial antibiotic discs was examined against all the tested bacterial isolates using the disc diffusion method of Kirby-Bauer

technique (Bauer, 1966). Using cotton swabs, Müller Hinton agar plates were uniformly inoculated with the tryptic soy broth of the bacterial cultures. Discs of antibiotics under test were loaded on the seeded plates by using sterile forceps. Inoculated plates were incubated at 37^{O}C for twenty-four hours, and then the inhibition zones were measured and expressed as the diameter of the inhibition zone (in mm) including the diameter of the paper disc.

Escherichia coli ATCC® 25922 and *Staphylococcus aureus* ATCC® 25923 were used as quality control, for Gram-negative and Gram-positive, strains for comparison with the tested isolates' susceptibility.

Antibiotics susceptibility testing for the bacterial isolates was evaluated according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2020). Table (1) summarizes the standard zone diameter for the bacterial susceptibility (S), intermediate (I), and resistance (R) cases against antibiotics under investigation.

Table 1. Interpretive categories and inhibition for *Enterobacteracae* and *Staphylococci* according to CLSI (CLSI, 2020)

Antibiotic		Inhibition zone diameter (mm)		Antibiotic		Inhibition zone diameter (mm)	
		Coliform	Staph			Coliform	Staph
AK-30	S[1]	≥ 17	≥ 26	**CEP-75**	S	≥ 34	≥ 29
Amikacin	I[2]	16-15	25-21	**Cefoperazone**	I	33-29	28-24
	R[3]	≤ 14	≤ 20		R	≤ 28	≤ 23
CN-10	S	≥ 15	≥ 15	**CFR-30**	S	≥ 27	≥ 31
Gentamycin	I	14-13	14-13	**Cefadroxil**	I	26-24	30-28
	R	≤ 12	≤ 12		R	≤ 23	≤ 27
VA-30	S	NA[4]	NA	**PRL-100**	S	≥ 30	≥ 30
Vancomycin	I	NA	NA	**Piperacillin**	I	29-25	29-25
	R	NA	NA		R	≤ 24	≤ 24
DO-30	S	≥ 24	≥ 16	**AMC-30**	S	NA	≥ 29
Doxycycline	I	23-19	15-13	**Amoxicillin**	I	NA	NA
	R	≤ 18	≤ 12		R	NA	≤ 28
DA-2	S	NA	≥ 21	**CTX-30**	S	≥ 26	≥ 31
Clindamycin	I	NA	20-15	**Cefotaxime**	I	25-23	30-26
	R	NA	≤ 14		R	≤ 22	≤ 25
AZM-15	S	NA	≥ 18	**SXT-25**	S	≥ 29	≥ 16
Azithromycin	I	NA	17-14	**Trimethoprim**	I	28-24	15-11
	R	NA	≤ 13	**/Sulphmethoxazole**	R	≤ 23	≤ 10

[1]S: susceptible [2]I: intermediate [3]R: resistance [4]NA: not assigned

Statistical analysis

Data were statistically analyzed using the SPSS program. Data are shown as mean ±standard deviation (SD) of three replicates. The t-test was done to compare means with the limits set by the Egyptian standard and p-value ≤ 0.05 was considered significant.

Results and Discussion

Microbiological analysis of Herring and Fesikh Samples

The microbiological examination is a core indicator for the evaluation of seafood quality. High microbial load indicates poor hygienic practices during manufacturing, handling, and storage of food. Furthermore, the poor practices contribute to the poor quality of the salted and smoked fish leading to off smell and physical damages for these products (Edris *et al.*, 2017).

Data presented in Table (2) and Fig. (2) showed the microbiological analysis of Herring and Fesikh samples. For Herring samples, the total viable counts ranged from 3.0 to 6.3 log CFU/g with mean value of 5.20 log CFU/g. As well, the total halophilic counts ranged from 2.0 to 6.0 log CFU/g with an average of 4.5 log CFU/g, while the aerobic spore former recorded values ranged from 1.0 to 3.0 log CFU/g with a mean of 1.4 log CFU/g. Whereas, bacterial counts of *E. coli* and *Staphylococcus aureus* were found to vary from 1.0 to 3.0 log CFU/g and from 3.0 to 5.8 log CFU/g, respectively with averages of 1.3 and 4.3 log CFU/g. Finally, the count of mold and yeast located in the range of 2.0-5.1 log CFU/g with an average of 4.3 log CFU/g.

Concerning the results of Fesikh samples (Table 2 and Fig. 2), the total viable count was ranged from 1.0 to 5.9 log CFU/g. Also, the total halophilic count ranged from 0.0 to 5.2 log CFU/g with a mean of 2.5±1.8 log CFU/g. While number means of anaerobic spore former bacteria was 2.1±1.2 log CFU/g with a value range of 0.0-3.0 log CFU/g. As well, *E. coli* and *Staphylococcus aureus* isolates recorded averages of 2.6±1.2 and 2.8±1.4 log CFU/g with values ranging from 1.0 to 4.0 and 1.0 to 4.5 log CFU/g, respectively. The mold and yeast recorded an average of 2.9±1.3 log CFU/g for values ranging from 1.0 to 4.6 log CFU/g.

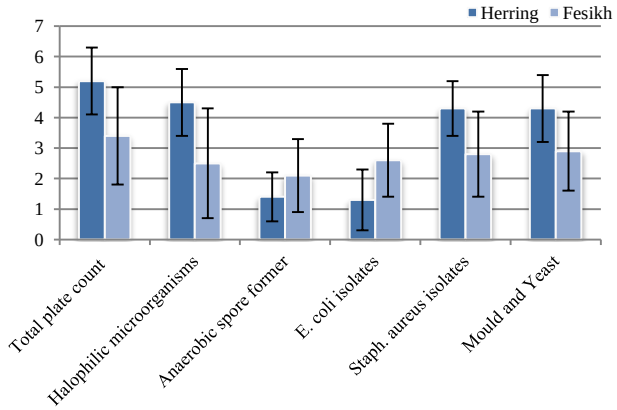


Fig. 2. Average microbial content of Herring and Fesikh samples

Table 2. Microbiological analysis of Herring and Fesikh Samples (log CFU/g)

Fish kind	Values	Total plate count	Halophilic microorganisms	Anaerobic spore former	*E. coli* isolates	*Staphylococcus aureus* isolates	Mold and Yeast
Herring	min	3.0	2.0	1.0	1.0	3.0	2.0
	max	6.3	6.0	3.0	3.0	5.8	5.1
Fesikh	min	1.0	0.0	0.0	1.0	1.0	1.0
	max	5.9	5.2	3.0	4.0	4.5	4.6

The total plate count (TPC) is one of the most important factors for determining the quality evaluation and overall acceptability of fish. TPC values present in Herring samples (3.0-6.3 log CFU/g) were higher than those found in Fesikh samples (1.0-5.9 log CFU/g). The average of current TPC value of Herring samples (5.2 log CFU/g) was in agreement with that obtained by Khater and Farag (2016). However, Edris *et al*. (2017) reported a lower mean of total aerobic plate count in Herring samples (4.17 log CFU/g),compared to the current findings. Meanwhile, Edris *et al*. (2014) found higher content of TPC (6.89 log CFU/g) in Fesikh samples. The higher microbial load in Herring samples may be attributed to the secondary contamination during handling, using contaminated ice and water as well as poor hygienic practices during processing, storage, and marketing (Edris *et al*., 2020).

The microbial load of anaerobic spore former in Herring and Fesikh samples was within mean values of 2.1 and 1.4 log CFU/g, respectively (Fig. 2). On the other hand, Khater and Farag (2016) observed a higher mean of anaerobic count (5.31 log CFU/g) in Herring samples than ours. The presence of anaerobic bacterial counts in Herring and Fesikh samples could be attributed to the cross-contamination through the used salt in fish. These bacteria may have survived during the smoking process (Khater & Farag, 2016). Also, the current results of halophilic bacteria were in agreement with a previous study conducted by Khater and Farag (2016) who reported that the average values of the halophilic bacteria in Egyptian Herring and Fesikh samples were 4.59 and 6.59 log CFU/g, respectively. Edris *et al*. (2017) found a comparable values of *E. coli*, *Staphylococcus aureus*, and mold and yeast in Egyptian Fesikh samples, accounting for 2.01, 1.58, and 1.22 log CFU/g, respectively.

Notably, the mean value of mold and yeast in Fesikh samples (2.9 log CFU/g) was lower than that of Herring samples (4.3 log CFU/g) (Fig. 2). The presence of mold and yeast could be attributed to the improper sanitation along the manufacturing process from catching to distributing and marketing. The contamination of smoked fish with molds increases the risk of infection with respective diseases as a result of mycotoxins production by some fungal strains (Edris, *et al*., 2017).

It is worth noting that average counts of different bacteria isolated from Herring samples were higher than those of Fesikh samples except for *E. coli* which was higher in Fesikh samples (2.6 log CFU/g), with no significant differences. In this regard, Khater and Farag (2016) reported similar viable counts of *E. coli* in Fesikh samples. However, El-Gazzar *et al*. (2020) reported a higher level (3.97 log CFU/g) of *E. coli* in Fesikh samples than those obtained in the present study. Also, the contamination of fish with *Enterobacteriaceae* could be associated with the formation of histamine as some species of *Enterobacteriaceae* can produce histamine enzymes during their growth (Björnsdóttir-Butler *et al*., 2010).

For the microbial load corresponds *Staphylococcus aureus* in samples, no significant differences were found between Herring samples (4.3 log CFU/g) and Fesikh samples (2.8 log CFU/g). Elkassas and Mousa (2021) reported comparable levels of *S. aureus* in Fesikh samples collected from Alexandria city, Egypt (3.45 log CFU/g). However, Edris *et al*. (2014) and Hassanien *et al*. (2016) reported higher counts of *S. aureus* 4.66 and 4.70 log CFU/g, respectively, in Fesikh samples.

It is worthy to mention that *Listeria monocytogenes* and *Vibrio parahaemolyticus* were not detected in all tested Herring and Fesikh samples collected in this study. This finding is in accordance with a previous study conducted by Hassanen *et al*. (2018) who reported the absence of *Listeria spp*. in smoked fish collected from markets in Menofiya, Egypt.

Those poor hygienic practices are likely the main responsible factor for the poor microbiological quality observed for Herring and Fesikh samples, especially in Sham El-Nessim occasion. So, Egyptian consumers may be at great risk of exposure to these pathogenic bacteria. Therefore, from season to season, isolated pathogens from Fesikh and Herring samples have to be tested for their susceptibility against antibiotics in the drug store. This will consequently highlight the most appropriate antibiotic for the clinical treatment of individuals exposed to these bacterial pathogens through the ingestion of contaminated fish.

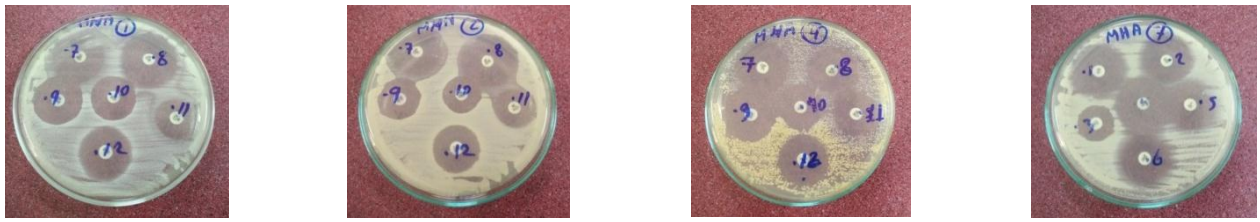
Antibiotic-susceptibility assessment for bacterial isolates

This study targeted the evaluation of antibiotics efficiency on inhibiting the growth of the different bacterial species isolated from Herring and Fesikh samples. Approximately 18 bacterial isolates were tested for their susceptibility against twelve antibiotics (Fig. 3). The susceptibility test was performed following the disc diffusion method of the Clinical Laboratory Standards for Antimicrobial Susceptibility Testing (CLSI, 2020). Data in Table (1) showed the standard zone diameter values of *E. coli* and *Staphylococcus aureus* isolates which were categorized into susceptible, intermediate, or resistant (S, I, or R) according to CLSI (CLSI, 2020). Notably, The CLSI document does not assign standard diameter values of inhibition zones for the total plate count (TPC), halophilic bacteria, and anaerobic bacteria. Therefore, it has been assumed that the highly effective antibiotics will be selected based on their higher values of inhibition zone against TPC, halophilic and anaerobic bacteria.

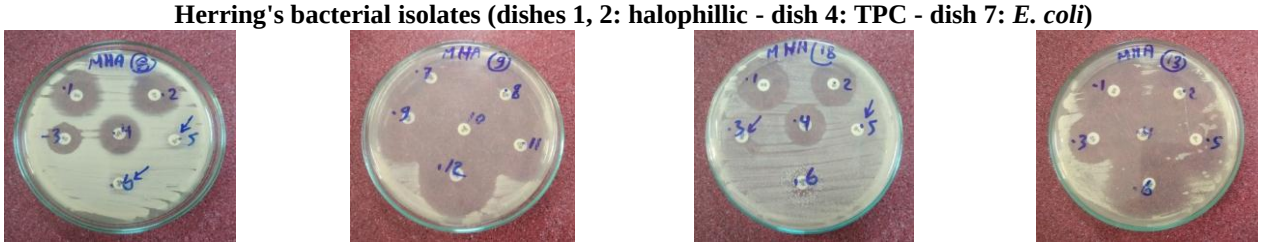
Data in Table (3a) showed values of inhibition zone for isolated bacteria from Herring samples as affected by the tested antibiotics. The results revealed that aminoglycoside antibiotics (AK-30 and CN-10) were found to be very effective against TPC in isolate 5 as 34.0 mm and 34.5 mm, respectively, and in isolate 4 were 28.5 mm and 25.0 mm, respectively. However, CFR-30 had no effect over TPC (isolate 5). Also, lincosamides (DA-2) failed to control total plate count (isolate 5). Halophilic bacteria showed a sensitivity response when tested by cephalosporine group (CEP-75 and CFR-30 µg) and gave zone diameters of 26.5 mm and 26.5 mm, respectively in isolate 1 and 28.0 mm and

31.0 mm, respectively in isolate 2. However, aminoglycoside (AK-30) appeared in the second-order as 24.0 mm in isolate 1 and 24.5 mm in isolate 2. Also, AK-30 and CN-10 scored the highest zone diameter value against anaerobic bacteria in isolate 15 as 34.0 mm and 34.5 mm, respectively, and in isolate 16 with zone diameters of 34.5±0.0[a] mm and 31.0±1.4[b] mm, respectively, with no significant differences with each of AZM-15 µg, CEP-75 µg, CFR-30 µg and PRL-100 µg.

The isolated *E. coli* and *Staphylococcus aureus* from Herring samples exhibited susceptibility (S) to aminoglycosides (AK-30 µg & CN-10 µg) as follows: 26.0 mm and 26.0 mm, respectively, for isolate 7 (*E. coli*), 29.0 mm and 29.0 mm, respectively, for isolate 10 (*Staphylococcus aureus*) and 31.5 mm and 31.5 mm, respectively, for isolate 11 (*Staphylococcus aureus*). Based on CLSI (CLSI, 2020) in Table (1), the standard inhibition zones of susceptibility (S) for *E. coli* and *Staphylococcus aureus* against amikacin (AK-30) were ≥17 mm and ≥ 26 mm, respectively, however for gentamycin (CN-10), it was ≥ 15 mm against both *E. coli* and *Staphylococcus aureus*. On the other hand, it was found that *E. coli* exhibited multi-resistance against all of the CEP-75 µg, PRL-100 µg, CTX-30 µg, and SXT-25 µg (Tables 1 and 3b). Noteworthy, the standard document of CLSI (2020) did not assign certain inhibition zones concerning S, I, and R cases for *E. coli* against many antibiotics (VA-30, DA-2, AZM-15, and AMC-30). Likewise, CLSI (2020) did not include the assessment of VA-30 µg against *Staphylococcus aureus*.



Herring's bacterial isolates (dishes 1, 2: halophillic - dish 4: TPC - dish 7: *E. coli*)



Fesikh's bacterial isolates (dishes 8, 9: *Staph. aureus* - dish 18: halophilic - dish 13: Anaerobic)

Fig. 3. Antibiotic-susceptibility test for bacterial isolates

Table 3a. Antibiotic sensitivity results for Herring's bacterial isolates (mean±SD)

Antibiotic group	Bacterial group (isolate codes)					
	TPC		Halophilic		Anaerob	
	4	5	1	2	15	16
Aminoglycosides						
AK-30 µg	28.5±0.7^{a}	34.0±1.4^{ab}	24.0±1.4^{bc}	24.5±0.7^{c}	34.0±1.4^{ab}	34.5±0.0^{a}
CN-10 µg	25.0±0.0^{bc}	34.5±0.7^{a}	21.0±1.4^{de}	22.0±0.0^{de}	34.5±0.7^{a}	31.0±1.4^{b}
Glycopeptides						
VA-30 µg	14.5±0.7^{f}	13.5±0.7^{g}	11.5±0.7^{g}	10.5±0.7^{g}	24.0±1.4^{ef}	24.0±0^{d}
Tetracyclines						
DO-30 µg	24.5±0.7^{bc}	27.5±0.7^{e}	22.5±0.7^{bcd}	21.0±0.0^{e}	31.0±1.4^{abc}	27.0±1.4^{c}
Lincosamides						
DA-2 µg	22.0±1.4^{de}	0.0±0.0^{h}	24.5±0.7^{ab}	23.0±0.0^{cd}	28.5±2.1^{cd}	27.5±0.7^{c}
Microlides						
AZM-15 µg	25.5±0.7^{bc}	32.0±1.4^{cd}	21.5±1.4^{cde}	19.0±1.15^{f}	33.0±0^{ab}	35.0±0^{a}
Cephalosporines						
CEP-75 µg	25.5±0.7^{b}	32.0±1.4^{bc}	26.5±0.7^{ab}	28.0±1.15^{b}	30.5±2.1^{bc}	34.0±0^{a}
CFR-30 µg	24.0±1.4^{bcd}	0.0±0.0^{h}	26.5±0.0^{a}	31.0±1.4^{a}	29.0±1.4^{c}	34.0±1.4^{a}
Beta-lactams						
PRL-100 µg	25.0±1.4^{bc}	29.0±1.4^{de}	24±0.7^{bc}	11.0±1.15^{g}	27.5±0.7^{cd}	35.0±0^{a}
AMC-30 µg	23.0±0.0^{cde}	19.0±1.4^{f}	21.5±0.0^{cd}	17.5±0.57^{f}	25.0± 0^{cd}	31.0±1.4^{b}
CTX-30 µg	21.0±1.4^{e}	27.5±0.7^{e}	20.0±0.0^{cde}	19.0±0.0^{f}	21.0±1.4^{f}	21.0±1.4^{e}
Sulfonamides						
SXT-25 µg	24.0±0.0^{bcd}	29.0±0.0^{de}	23.5±0.57^{bc}	21.50±0.57^{de}	28.0±0^{cd}	29.0±1.4^{c}

AK-30: Amikacin, **CN-10:** Gentamycin, **VA-30:** Vancomycin, **DO-30:** Doxycycline, **DA-2:** Clindamycin, **AZM-15:** Azithromycin, **CEP-75:** Cefoperazone, **CFR-30:** Cefadroxil, **PRL-100:** Piperacillin, **AMC-30:** Amoxicillin, **CTX-30:** Cefotaxime, **SXT-25:** Trimethoprim /Sulphmethoxazole

Values with the same letter have no significant difference among them.

Table 3b. Antibiotic sensitivity results for Herring's bacterial isolates (mean±SD)

Antibiotic group	Bacterial group (isolate codes)						Standard strains			
	E. coli isolates		*Staph. aureus* isolates				*E. coli* ATCC® 25922		*S. aureus* ATCC® 25923	
	7		10		11					
Aminoglycosides										
AK-30 µg	26.0±1.4	**S**[1]	29.0±1.4	**S**	31.5±2.1	**S**	17.5±0.7	**S**	23.5±0.7	**I**
CN-10 µg	26.0±0.0	**S**	29.0±0.0	**S**	31.5±0.7	**S**	16.0±0.0	**S**	21.5±0.7	**S**
Glycopeptides										
VA-30 µg	14.0±1.4	**NA**[2]	17.0±1.4	**NA**	28.0±1.4	**NA**	08.5±0.7	**NA**	14.5±0.7	**NA**
Tetracyclines										
DO-30 µg	24.5±0.7	**S**	28.0±0.0	**S**	33.5±2.1	**S**	11.5±0.7	**R**	26.0±0.0	**S**
Lincosamides										
DA-2 µg	22.0±1.4	**NA**	25.5±0.7	**S**	14.5±0.7	**R**	08.0±0.0	**NA**	13.5±0.7	**R**
Microlides										
AZM-15 µg	21.5±2.1	**NA**	26.0±0.0	**S**	34.0±1.4	**S**	16.5±0.7	**NA**	22.0±0.0	**S**
Cephalosporines										
CEP-75 µg	26.0±1.4	**R**[3]	34.5±0.7	**S**	34.5±0.7	**S**	25.0±0.0	**R**	26.0±1.4	**I**[4]
CFR-30 µg	26.5±0.7	**I**	34.5±0.7	**S**	34.5±0.7	**S**	17.0±0.0	**R**	28.5±0.7	**I**
Beta-lactams										
PRL-100 µg	24.0±1.4	**R**	28.5±0.7	**I**	28.5±0.7	**I**	20.5±0.7	**R**	24.0±1.4	**R**
AMC-30 µg	23.5±0.7	**NA**	31.5±0.7	**S**	31.5±0.7	**S**	15.5±0.7	**NA**	24.0± 0.0	**R**
CTX-30 µg	20.5±0.7	**R**	30.0±0.0	**I**	30.0± 0.0	**I**	22.5±0.7	**R**	20.5±0.7	**R**
Sulfonamides										
SXT-25 µg	21.0±1.4	**R**	27.5±0.7	**S**	27.5±0.7	**S**	24.0±0.0	**I**	23.5±0.7	**S**

AK-30: Amikacin, **CN-10:** Gentamycin, **VA-30:** Vancomycin, **DO-30:** Doxycycline, **DA-2:** Clindamycin, **AZM-15:** Azithromycin, **CEP-75:** Cefoperazone, **CFR-30:** Cefadroxil, **PRL-100:** Piperacillin, **AMC-30:** Amoxicillin, **CTX-30:** Cefotaxime, **SXT-25:** Trimethoprim /Sulphmethoxazole

[1]S: susceptible [2]NA: not assigned [3]R: resistant [4]I: intermediate

Values with the same letter have no significant difference among them.

Escherichia coli ATCC® 25922 and *Staphylococcus aureus* ATCC® 25923 were used as comparative or quality control strains, for the isolated *E. coli* and *Staphylococcus aureus* from Herring and Fesikh samples, concerning the susceptibility pattern against antibiotics. *Escherichia coli* ATCC® 25922, gram-negative control strain, was susceptible against aminoglycosides; AK-30 and CN-10 antibiotics, presenting inhibition zone diameters of 17.5 mm and 16.0 mm, respectively, (CLSI, 2020). On the other hand, this control strain showed a multi-resistance pattern to all of tetracyclines (DO-30 µg), cephalosporines (CEP-75 µg and CFR-30 µg), and beta-lactams (PRL-100 µg and CTX-30 µg). Only, SXT-25 µg had an intermediate effect (I) against *Escherichia coli* ATCC® 25922 (24.0 mm). As mentioned above, the CLSI (2020) does not involved any susceptibility assessment of VA-30 µg, DA-2 µg, AZM-15 µg and AMC-30 µg, against *Escherichia coli.*

Staphylococcus aureus ATCC® 25923, gram-positive control strain, was susceptible (S), according to CLSI (2020), to each of CN-10 µg, DO-30 µg, AZM-15 µg, and SXT-25 µg, presenting inhibition zones of 21.5, 26.0, 22.0, and 23.5 mm, respectively. On the contrary, *Staphtlococcus aureus* ATCC® 25923, was resistant (R) against lincosamides (DA-2 µg) and beta-lactams (PRL-100 µg, AMC-30 µg, and CTX-30 µg). While it showed intermediate (I) response to AK-30 µg from aminoglycosides group and to both of CEP-75 µg and CFR-30 µg from cephalosporines group. Notably, *E. coli* isolates of Herring samples had the same susceptibility pattern as the control strain (*E. coli* ATCC® 25922). On the other hand, the control strain of *Staphylococcus aureus* ATCC® 25923 was more resistant to the studied antibiotics as compared to the isolated strains of *Staph. aureus* from Herring samples.

concerning the susceptibility *E. coli* against antibiotics, The obtained results were in general agreement with the results obtained by Adenaike *et al*. (2016) who confirmed the susceptibility of *E. coli* isolated from smoked fish to amikacin antibiotic (aminoglycoside group). Also, the current results were in line with those obtained by Ryu *et al*. (2012) and Gufe, *et al*. (2019) who reported that *E. coli* isolated from fish showed a multi-resistance pattern against tetracyclines, cephalosporins, and beta-lactams antibiotics. Furthermore, in the study carried out by Hassanen *et al*. (2018), it was reported that *E. coli* isolated from smoked fish in Egypt had a multi-resistance pattern to some antibiotics like cefozon (cephalosporin), gentamicin (aminoglycoside), cefotaxime (cephalosporin), doxycycline (tetracycline), and clindamycin (lincomycin).

As can be seen in Tables (3a and 3b), it could be concluded that the most effective antibiotics against the majority of tested microbes isolated from Herring samples can be arranged as aminoglycosides > cephalosporines > tetracyclines.

Concerning the susceptibility results of bacterial isolates of Fesikh samples, Table (4a) showed that CFR-30 µg, Ak-30 µg, Do-30 µg, and CEP-75 µg had the highest effect against TPC in isolate 6, with no significant differences. For halophilic bacteria, it was found that aminoglycoside (AK-30) was highly effective giving inhibition zones of 31.0 mm and 34.0 mm, respectively, for isolates 3 and 17. However, CFR-30 had no control over isolate 3 as 0.0±0.0[g] mm. AK-30 µg, CN-10 µg, CEP-75 µg, and CFR-30 µg were found to be very effective against anaerobic bacteria in isolate 14 giving inhibition zones of 31.0 mm, 30.5 mm, 31.0 mm, and 31.5 mm, respectively, with no significant differences.

Table 4a. Antibiotic sensitivity results for Fesikh's bacterial isolates (mean±SD)

Antibiotic group	Bacterial group (isolate codes)					
	TPC	Halophilic			Anaerob	
	6	3	17	18	13	14
Aminoglycosides						
AK-30 µg	30.0±1.4^{ab}	31.0±1.4^{a}	34.0±1.4^{a}	24.0±1.4^{c}	33.5±0.7^{a}	31.0±1.4^{ab}
CN-10 µg	27.5±2.1^{bc}	29.0±1.4^{a}	31.0±1.4^{bcd}	22.0±0^{d}	25.0±0^{def}	30.5±0.7^{ab}
Glycopeptides						
VA-30 µg	17.0±1.4^{g}	17.5±3.5^{d}	19.0±1.4^{g}	9.0±1.4^{h}	23.0±1.4^{f}	21.0±1.4^{f}
Tetracyclines						
DO-30 µg	30.0±0.0^{ab}	26.0±1.4^{c}	28.0±0d^{e}	19.0±1.4^{e}	27.0±1.4^{cd}	28.0±1.4^{bcde}
Lincosamides						
DA-2 µg	23.0±1.4^{de}	15.5±2.1^{d}	25.0±0^{f}	0.0±0^{i}	12.5±0.7^{g}	26.5±0.7^{e}
Microlides						
AZM-15 µg	25.0±1.4^{cd}	29.0±1.4^{a}	33.0±1.4^{ab}	8.5±0.7^{h}	29.5±0.7^{b}	30.0±1.4^{abc}
Cephalosporines						
CEP-75 µg	29.0±1.4^{ab}	28.5±2.1^{b}	32.5±0.7^{ab}	30.5±0.7^{a}	26.5±0.7^{cde}	31.0±1.4^{ab}
CFR-30 µg	31.0±1.4^{a}	0.0±0.0^{g}	32.0±0^{abc}	15.5±0.7^{f}	28.0±0^{bc}	31.5±2.1^{a}
Beta-lactams						
PRL-100 µg	28.0±0.0^{abc}	9.0±1.4^{ef}	29.5±0.7^{cde}	28.0±0^{b}	25.0±0^{def}	29.5±0.7^{abcd}
AMC-30 µg	21.0±1.4^{ef}	11.5±2.1^{e}	31.5±2.1^{defg}	11.0±1.4^{g}	24.5±0.7^{ef}	27.0±1.4^{de}
CTX-30 µg	18.5±2.1^{fg}	17.5±3.5^{d}	21.5±0.7^{g}	20.0± 0^{e}	26.5±0.7^{cde}	22.5±0.7^{f}
Sulfonamides						
SXT-25 µg	21.5±0.7^{ef}	19.5±2.1^{d}	29.0±1.4^{cd}	8.0±0^{h}	34.0±1.4^{a}	27.5±0.7^{cde}

AK-30: Amikacin, **CN-10:** Gentamycin, **VA-30:** Vancomycin, **DO-30:** Doxycycline, **DA-2:** Clindamycin, **AZM-15:** Azithromycin, **CEP-75:** Cefoperazone, **CFR-30:** Cefadroxil, **PRL-100:** Piperacillin, **AMC-30:** Amoxicillin, **CTX-30:** Cefotaxime, **SXT-25:** Trimethoprim /Sulphmethoxazole

Values with the same letter have no significant difference among them.

Table 4b. Antibiotic sensitivity results for Fesikh's bacterial isolates (mean±SD)

Antibiotic group	Bacterial group (isolate codes)						Standard strains			
	E. coli isolates		*Staph. aureus* isolates				*E. coli* ATCC® 25922		*S. aureus* ATCC® 25923	
	12		8		9					
Aminoglycosides										
AK-30 µg	31.50±0.5	**S**[1]	23.5±0.7	**I**[2]	34.0±1.4	**S**	17.5±0.7	**S**	23.5±0.7	**I**
CN-10 µg	31.00±1.0	**S**	20.5±0.7	**S**	31.0±1.4	**S**	16.0±0.0	**S**	21.5±0.7	**S**
Glycopeptides										
VA-30 µg	26.00±1.0	**NA**[3]	14.0±1.4	**NA**	21.0±1.4	**NA**	08.5±0.7	**NA**	14.5±0.7	**NA**
Tetracyclines										
DO-30 µg	29.00±1.0	**S**	18.0±1.4	**S**	32.5±0.7	**S**	11.5±0.7	**R**[4]	26.0±0.0	**S**
Lincosamides										
DA-2 µg	15.00±1.0	**NA**	08.5±0.7	**R**	27.5±0.7	**S**	08.0±0.0	**NA**	13.5±0.7	**R**
Microlides										
AZM-15 µg	28.50±1.5	**NA**	9.0±1.4	**R**	26.0±1.4	**S**	16.5±0.7	**NA**	22.0±0.0	**S**
Cephalosporines										
CEP-75 µg	31.00±1.0	**S**	19.0±1.4	**R**	34.5±0.7	**S**	25.0±0.0	**R**	26.0±1.4	**I**
CFR-30 µg	32.00±1.0	**S**	23.0±0.0	**R**	34.0±1.4	**S**	17.0±0.0	**R**	28.5±0.7	**I**
Beta-lactams										
PRL-100 µg	27.00±1.0	**I**	21.0±0.0	**R**	27.0±1.4	**I**	20.5±0.7	**R**	24.0±1.4	**R**
AMC-30 µg	27.00±1.0	**NA**	19.5±0.7	**R**	31.0±1.4	**S**	15.5±0.7	**NA**	24.0±0.0	**R**
CTX-30 µg	24.50±0.5	**I**	18.0±0.0	**R**	30.5±0.7	**I**	22.5±0.7	**R**	20.5±0.7	**R**
Sulfonamides										
SXT-25 µg	24.00±1.0	**I**	11.0±1.4	**I**	31.0±1.4	**S**	24.0±0.0	**I**	23.5±0.7	**S**

AK-30: Amikacin, **CN-10:** Gentamycin, **VA-30:** Vancomycin, **DO-30:** Doxycycline, **DA-2:** Clindamycin, **AZM-15:** Azithromycin, **CEP-75:** Cefoperazone, **CFR-30:** Cefadroxil, **PRL-100:** Piperacillin, **AMC-30:** Amoxicillin, **CTX-30:** Cefotaxime, **SXT-25:** Trimethoprim /Sulphmethoxazole

[1]S: susceptible [2]I: intermediate [3]NA: not assigned [4]R: resistant

Values with the same letter have no significant difference among them.

Escherichia coli (isolate 12) from Fesikh samples in Table (4b) exhibited that susceptibility (S) values against AK-30 µg, CN-10 µg, DO-30 µg, CEP-75 µg, and CFR-30 µg gave inhibition zones of 31.50 mm, 31.00 mm, 29.00 mm, 31.00 mm and 32.00 mm, respectively, according to CLSI (2020) in Table (1). However, PRL-100 µg, CTX-30 µg, and SXT-25 µg had an intermediate (I) effect against *Escherichia coli* isolate giving inhibition zones of 27.00 mm, 24.50 mm, and 24.00 mm, respectively. While, values of inhibition zones resulted by using VA-30 µg, DA-2 µg, AZM-15 µg, and AMC-30 µg were not included in CLSI (2020). *Escherichia coli* ATCC® 25922 as a gram-negative control strain

exhibited a different response pattern as compared to the isolated *Escherichia coli* from Fesikh samples. In particular, *E. coli* ATCC® 25922 had a multi-resistance (R) effect to most antibiotics; DO-30 µg, CEP-75 µg, CFR-30 µg, PRL-100 µg, and CTX-30 µg. As well, only SXT-25 µg had an intermediate (I) effect, while all of VA-30 µg, DA-2 µg, AZM-15 µg, and AMC-30 µg were not assigned in CLSI (2020). Meanwhile, the control strain of *E. coli* showed a susceptibility (S) response towards AK-30 µg and CN-10 µg (17.5 mm and 16.0 mm, respectively) according to the CLSI (2020).

Staphylococcus aureus of isolate no. (8) showed resistance (R) pattern to most of the tested antibiotics such as DA-2 µg, AZM-15 µg, CEP-75 µg, CFR-30 µg, PRL-100 µg, AMC-30 µg and CTX-30 µg (Table 4b). However, this isolate showed an intermediate (I) response to AK-30 µg and SXT-25 µg with inhibition zones of 23.5 mm and 11.0 mm, respectively. Only CN-10 µg and DO-30 µg induced a susceptible (S) effect against isolate 8. Interestingly, isolate no. (9) of *Staphylococcus aureus* was susceptible (S) to most antibiotics under the study. However, only PRL-100 µg and CTX-30 µg had an intermediate (I) response from isolate no. (9) with zone diameters of 27.0 ± 1.4^{c} mm and 30.5 ± 0.7^{b} mm, respectively. *Staphylococcus aureus* ATCC® 25923 as a gram-positive control strain had a susceptibility (S) effect, according to the protocol of CLSI (2020), to each of CN-10 µg, DO-30 µg, AZM-15 µg and SXT-25 µg as 21.5±0.7 mm, 26.0±0 mm, 22.0±0 mm and 23.5±0.7 mm, respectively. However, DA-2 µg, PRL-100 µg, AMC-30 µg and CTX-30 µg had a resistance (R) effect. While, AK-30 µg, CEP-75 µg and CEP-75 µg produced an intermediate (I) effect to *Staph. aureus* ATCC® 25923.

These results were in agreement with the data obtained by Imarhiagbe *et al*. (2016) who reported that *Staphylococcus* sp. can develop multi-resistance against many antimicrobial agents which causes health problems. Furthermore, the results confirmed that seafood and commercial fish may act as a reservoir for multi-resistant bacteria (Ryu, *et al*., 2012).

Interestingly, based on the obtained results in Tables (3 and 4), the tested classes of antibiotics can be arranged, based on their antibacterial effect on Fesikh's bacterial isolates, as follows: aminoglycosides ≥ cephalosporines > tetracyclines > sulfonamides.

Conclusion

According to the results of the present study, Herring and Fesikh samples sold in Cairo and Alexandria, at Sham El-Nessim's occasion of 2021, were contaminated with pathogenic bacteria. Also, based on the results of the antibiotic-susceptibility test, antibiotic classes can be arranged, according to their effectiveness against bacterial isolates, as follows: aminoglycosides ≥ cephalosporines > tetracyclines. In conclusion, aminoglycoside antibiotics such as amikacin (AK-30 µg) and gentamycin (CN-10 µg) have proved their effectiveness in inhibiting the growth of isolated bacteria from Herring and Fesikh. Accordingly, the obtained results in the present investigation recommended the use of aminoglycoside group as the first line of defense to treat the potential foodborne illness originated from the consumption of contaminated Herring and Fesikh in Sham El-Nessim's occasion.

Noteworthy, it is highly recommended to reduce the use of antibiotics as much as possible. As well, the use of narrow-spectrum antibiotics is highly recommended rather than broad-spectrum antibiotics to limit the prevalence of antibiotic resistance. Additionally, continuous surveillance is recommended, to test the susceptibility of isolated pathogens from fish and seafood, to ensure the safety of these fish products. As well, such evaluation data will be of help with physicians in the prescription of appropriate antibiotics to treat individuals exposed to pathogenic bacteria from such kind of fish. Moreover, this susceptibility assessment data could be used by the decision-makers for the procedures of control and/or treatment of foodborne outbreaks from seafood or other food types.

References

11290-1:2017, I. (2017). Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of *Listeria spp*. — Part 1: Detection method. .

21872-1:2017-06, I. (2017). Microbiology of the food chain — Horizontal method for the determination of Vibrio spp. — Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*.

Adenaike, O., Olonitola, O., Ameh, J. & Whong, C. (2016). Multidrug resistance and multiple antibiotic resistance index of *Escherichia coli* strains isolated from retailed smoked fish. J. Nat. Sci. Res 6: 2225-2921

Adesoji, A. T., Onuh, J. P., Musa, A. O. & Akinrosoye, P. F. (2019). Bacteriological qualities and antibiogram studies of bacteria from "suya" and smoked fish (*Clarias gariepinus*) in Dutsin-Ma, Katsina State, Nigeria. Pan Afr. Med. J. 33: 219-219.DOI: 10.11604/pamj.2019.33.219.17729

Ahmed, M. B. M., Abdel-Rahman, G. N., Salem, S. H. & Fouzy, A. S. M. (2020). Incidence, stability and risk assessment for sulfonamides and tetracyclines in aqua-cultured Nile Tilapia fish of Egypt. Toxicol. Rep. 7: 836-843.DOI: https://doi.org/10.1016/j.toxrep.2020.06.009

Al-Busaidi, M. A., Jukes, D. J. & Bose, S. (2016). Seafood safety and quality: An analysis of the supply chain in the Sultanate of Oman. Food Control 59: 651-662.DOI: https://doi.org/10.1016/j.foodcont.2015.06.023

Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45: 149-158

Björnsdóttir-Butler, K., Bolton, G. E., Jaykus, L.-A., McClellan-Green, P. D. & Green, D. P. (2010). Development of molecular-based methods for determination of high histamine producing bacteria in fish. Int. J. Food Microbiol. 139(3): 161-167.DOI: https://doi.org/10.1016/j.ijfoodmicro.2010.03.017

CLSI, C. a. L. S. I. (2020). Performance Standards for Antimicrobial Susceptibility Testing.

Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., Nakayama, S. & Ishizuka, M. (2013). Antibiotic residues in food: the African scenario. Jpn. J. Vet. Res. 61(Supplement): S13-S22

Dobiasova, H., Kutilova, I., Piackova, V., Vesely, T., Cizek, A. & Dolejska, M. (2014). Ornamental fish as a source of plasmid-mediated quinolone resistance genes and antibiotic resistance plasmids. Vet. Microbiol. 171(3): 413-421.DOI: https://doi.org/10.1016/j.vetmic.2014.02.011

Edris, A., Amin, R. A., Naseif, M. Z. & AbdelFatah, E. M. (2014). Evaluation of retiled salted fish according to Egyptian Standard. Benha Vet. Med. J. 27(2): 168-176.DOI: https://www.bvmj.bu.edu.eg/issues/27-2/16.pdf

Edris, A. M., Hassanien, F. S., Shaltout, F. A. E., ELbaba, A. H. & Adel, N. M. (2017). Microbiological evaluation of some heat treated fish products in Egyptian markets. Benha Vet. Med. J. 33(2): 305-316.DOI: 10.21608/bvmj.2017.30491

Edris, A. M., Tolba, K. S., Meky, H. H. & Meky, M. A. (2020). Quality and safety evaluation of salted fish. J. Vet. Anim. Res. 3(2): 1-6.DOI: http://article.scholarena.com/Quality-and-Safety-Evaluation-of-Salted-Fish.pdf

El-Gazzar, E., Hassanien, F. & Abou ELRoos, N. A. (2020). Bacterial hazards of ready to eat fish products. Benha Vet. Med. J. 39(2): 10-14.DOI: 10.21608/bvmj.2020.25463.1181

Elbashir, S., Parveen, S., Schwarz, J., Rippen, T., Jahncke, M. & DePaola, A. (2018). Seafood pathogens and information on antimicrobial resistance: a review. Food Microbiol. 70: 85-93.DOI: https://doi.org/10.1016/j.fm.2017.09.011

Elkassas, W. M. & Mousa, H. M. (2021). Correlation between Methicillin resistance and enterotoxins production in *Staphylococcus aureus* isolated from some salted fish. Alex. J. Vet. Sci. 70(1).DOI: 10.5455/ajvs.92152

Feldhusen, F. (2000). The role of seafood in bacterial foodborne diseases. Microbes and Infection 2(13): 1651-1660.DOI: https://doi.org/10.1016/S1286-4579(00)01321-6

Gassem, M. A. (2019). Microbiological and chemical quality of a traditional salted-fermented fish (Hout-Kasef) product of Jazan region, Saudi Arabia. Saudi J. Biol. Sci. 26(1): 137-140.DOI: https://doi.org/10.1016/j.sjbs.2017.04.003

Gufe, C., Canaan Hodobo, T., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, P. V. & Machakwa, J. (2019). Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe. Int. J. Microbiol. 2019: 8759636.DOI: 10.1155/2019/8759636

Hassanen, F. S., Sabike, I. I., Haikel, G. I. & Elged, E. A. (2018). Screening for antimicrobial resistance in some pathogens isolated from cold smoked fish marketed in Menofiya Government. Benha Vet. Med. J. 35(2): 269-283.DOI: 10.21608/bvmj.2018.96302

Hassanien, F. S., Hassan, M. A., Shawkey, N. & A., A. E. (2016). Enterotoxin producing *S. aureus* in salted fish. Benha Vet. Med. J. 31(1): 30-34.DOI: 10.21608/bvmj.2016.31214

Hosseini, H., Cheraghali, A. M., Yalfani, R. & Razavilar, V. (2004). Incidence of *Vibrio spp*. in shrimp caught off the south coast of Iran. Food Control 15(3): 187-190.DOI: https://doi.org/10.1016/S0956-7135(03)00045-8

Imarhiagbe, E., Ofuya, M., Osarenotor, O. & Ekhaise, F. (2016). Antibiotic sensitivity pattern of microorganisms isolated from smoked and frozen fishes sold in Benin and Warri metropolis. Niger. J. Biotechnol. 31: 40-45.DOI: 10.4314/njb.v31i1.6

Iwamoto, M., Ayers, T., Mahon, B. E. & Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. Clin. Microbiol. Rev. 23(2): 399-411.DOI: doi:10.1128/CMR.00059-09

Khater, D. F. & Farag, S. E.-S. (2016). Evaluation of bacterial and chemical quality of new manufactured pasted fish products in a large scale fish processing plant, Egypt. Benha Vet. Med. J. 31(2): 63-72.DOI: 10.21608/bvmj.2016.31263

Kümmerer, K. (2009). Antibiotics in the aquatic environment – a review – Part I. Chemosphere 75(4): 417-434.DOI: https://doi.org/10.1016/j.chemosphere.2008.11.086

Marti, E., Variatza, E. & Balcazar, J. L. (2014). The role of aquatic ecosystems as reservoirs of antibiotic resistance. Trends Microbiol. 22(1): 36-41.DOI: 10.1016/j.tim.2013.11.001

Mizan, M. F. R., Jahid, I. K. & Ha, S.-D. (2015). Microbial biofilms in seafood: A food-hygiene challenge. Food Microbiol. 49: 41-55.DOI: https://doi.org/10.1016/j.fm.2015.01.009

Nguyen, H. N. K., Van, T. T. H., Nguyen, H. T., Smooker, P. M., Shimeta, J. & Coloe, P. J. (2014). Molecular characterization of antibiotic resistance in *Pseudomonas* and *Aeromonas* isolates from catfish of the Mekong Delta, Vietnam. Vet. Microbiol. 171(3): 397-405.DOI: https://doi.org/10.1016/j.vetmic.2014.01.028

Rodriguez-Mozaz, S., Chamorro, S., Marti, E., Huerta, B., Gros, M., Sànchez-Melsió, A., Borrego, C. M., Barceló, D. & Balcázar, J. L. (2015). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Res. 69: 234-242.DOI: https://doi.org/10.1016/j.watres.2014.11.021

Ryu, S.-H., Park, S.-G., Choi, S.-M., Hwang, Y.-O., Ham, H.-J., Kim, S.-U., Lee, Y.-K., Kim, M.-S., Park, G.-Y., Kim, K.-S. & Chae, Y.-Z. (2012). Antimicrobial resistance and resistance genes in *Escherichia coli* strains isolated from commercial fish and seafood. Int. J. Food Microbiol. 152(1): 14-18.DOI: https://doi.org/10.1016/j.ijfoodmicro.2011.10.003

Samanidou, V. F. & Evaggelopoulou, E. N. (2007). Analytical strategies to determine antibiotic residues in fish. J. Sep. Sci. 30(16): 2549-2569.DOI: https://doi.org/10.1002/jssc.200700252

Sharma, V. K., Johnson, N., Cizmas, L., McDonald, T. J. & Kim, H. (2016). A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere 150: 702-714.DOI: https://doi.org/10.1016/j.chemosphere.2015.12.084

Yang, X., Wu, Q., Zhang, J., Huang, J., Chen, L., Liu, S., Yu, S. & Cai, S. (2015). Prevalence, enumeration, and characterization of *Salmonella* isolated from aquatic food products from retail markets in China. Food Control 57: 308-313.DOI: https://doi.org/10.1016/j.foodcont.2015.03.046

Zhabiz, G., Omar, B. & Donald Gene, P. (2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J. Infect. Dev. Ctries 8(02).DOI: 10.3855/jidc.3573