Isolation and Resistance to Antibiotics of Total Coliforms of Lisa Fish (*Mugil Incilis*) from Cartagena de Indias Bay, Colombia

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Abstract—The emergence of antibiotic resistant bacteria has been increasing, these have been appearing in wild animals for different reasons. For this reason, it is essential to determine whether the total isolated of lisa (*Mugil incilis*) coliforms captured from the Cartagena de Indias bay (Colombia) show resistance to different antibiotics. Lisas were fished in the Cartagena bay and then taken to the microbiology laboratory of the Engineering Faculty of the University of Cartagena, after which the bacteria were isolated from the gills and intestines. The determination of total coliforms was carried out using the MPN method. Of the isolated microorganisms (*E. coli* and *Enterobacter aerogenes*) the highest proportion showed resistance to the antibiotics evaluated, it was also observed that the highest susceptibility occurred against chloramphenicol, ceftriaxone and amikacin. Given the results, the high degree of pollution of the bay's waters is confirmed, in addition to the fact that the microorganisms showed resistance to the evaluated antibiotics and in some cases the same microorganism was resistant to more than two antibiotics.

Keywords: antibiotic, multiresistance, microorganism, Caribbean Sea, MPN.

I. INTRODUCTION

Fish meat is perceived as a healthy food and an alternative to other types of meat, such as red meats [1] and in turn plays a fundamental role in feeding the world's population, as it contributes a significant part of the protein requirement in many regions [2], In addition, these proteins are of excellent quality, they also provide vitamins and minerals and have a unique and healthier lipid profile than other protein foods, such as beef [3].

Members of the *Mugilidae* family, generally known as smooth, are coastal marine fish with a worldwide distribution, found at all temperatures. Fish of this family not only inhabit coastal waters, but also, depending on the species, spend part or even all of their life cycle in coastal lagoons, lakes and/or rivers [4]. *Mugil incilis* is a species of great importance in the diet of the Colombian Caribbean region, because it is a species that inhabits this area [5], occurs at all times of the year and is part of traditional foods.Total Coliforms are a wide variety of optional aerobic and anaerobic bacilli, gram negative and non-sporulant. The coliform group includes genus of bacteria such as *Escherichia*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Serratia* and Hafnia [6], much of the total Coliforms are present in the environment, soil, vegetation and decomposing matter [7]. This group of bacteria is used as an indicator of water quality for domestic or industrial use and food products [8].

Antibiotics are like compounds that can effectively inhibit the growth of microorganisms [9]. These can be divided into two groups based on their effect on microbial cells through two main mechanisms, which are bactericidal that kill bacteria or bacteriostatic, which suppress the growth of bacteria (Table 1) [10].

Table 1. Classification of antibiotics by bacterial effect

Bactericide	Bacteriostatic
Penicillin	Macrolide
Cephalosporin	Tetracycline
Aminoglycoside	Doxycycline
Rifampicin	Lincosamide
Quinolone	Chloramphenicol
Monobactamicin	Erythromycin
Fluoroquinolone	Sulfonamide

Source: 11, 12

The irrational use of antibiotics is the main driving force behind the emergence and spread of resistant bacteria [13, 14]. This misuse has led to the development of new resistance mechanisms, which jeopardizes the effective treatment of common infectious diseases [15],it has also led to cases of multidrug-resistance to antibiotics from bacteria such as E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa [16].

Recently, some authors [17, 18], reported the presence of residual antibiotics in wild fish meat samples. The bacterial contamination suffered by fish comes from the native flora that exists in the oceans and seas where they live, the vast majority of which corresponds to Gram-negative bacteria, including microorganisms belonging to the genus *Pseudomonas*, *Alteromonas*, *Moraxella*, *Acinetobacter*, *Vibrio* and *Flavobacterium*, however, in tropical climates, these are accompanied by Gram-positive mesophilic bacteria belonging mostly to Micrococcus and Bacillus [19]. The bacterial flora in freshly caught fish depends on the capture environment, with very high counts of for example 10^7 CFU/cm^2 in fish caught in highly polluted waters [20]. Therefore, the objective of this research was to determine antibiotic resistance of total Coliforms isolated from smooth fish (*Mugilincilis*) in the Cartagena de Indiasbay(Colombia).

II. METHODOLOGY

A. Sample collection and transport

The mullets were collected directly from Cartagena Bay with the help of local fishermen during the month of June on days 2 (M1), 8 (M2), 15 (M3), 17 (M4) and 23 (M5). Once captured, the mullets were stored in hermetically sealed plastic bags (Ciplox bags) and transported in ice coolers to the microbiology laboratory of the Engineering Faculty of the University of Cartagena.

B. Isolation of bacteria

To isolate the bacteria, 50 g of the gills and smooth intestines were weighed and then mixed with 450 mL of sterile peptonated water. It was then homogenized in an Oster blender. All isolates were characterized by gram staining and biochemical testing [21].

C. Determination of Total Coliforms

The determination of total coliforms was made by the most probable Number (MPN). Serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} of the mixture previously made were performed. 1 mL of each dilution was taken to inoculate in bright green bile broth (Caldobrila) (Merck Germany) and incubated at 37°C for 24 hours. The tubes in which there was evidence of oxygen in the Ducan bell were considered positive, these were planted Eosin Methylene Blue (EMB) agar (MercK Germany). After 24 hours of incubation, Gram staining and biochemical tests were carried out according to the methodology used by Pascual and Calderón [22].Quality control and validation of susceptibility patterns were performed with the Escherichia coli reference strain ATCC 35218 (American Type Culture Collection. Rockville, USA).

D. Evaluation of susceptibility to antibiotics

This was done according to Kirby-Bauer's methodology [23]. The susceptibility to antibiotics was realized in agar diffusion Muller Hinton of the Oxoid brand (UK) in previous bacterial suspension corresponding to an optical density (DO) of 0.5 according to the standard scale of McFarland, corresponding to 10^8 CFU mL^{-1} and diluted 1:100 in saline solution 0.85%. The antibiotic discs evaluated were: Amikacin (AN), Ampicillin (AM), Cetriaxone (CRO), Oxacillin (OX), Chloramphenicol (C) Sulfametoxazole (STX) and Ceftacidime (CTX) from Oxoid (UK). Petri dishes were incubated at 35°C for 24 h and the diameters of each antibiotic's inhibition halo were measured. The determination with each antibiotic was performed in duplicate and each microorganism was classified according to inhibition diameter into: sensitive, moderately sensitive or resistant according to National Committee for Clinical Laboratory Standardization (NCCLS) standards. Later, the two antibiotics that had more resistance from the bacteria were taken and were mixed with each other of the antibiotics used in this research.

E. Statistical analysis

The data were subjected to the ANOVA test, to compare the mean value of two or more groups and to determine the statistical significance. In this case the total coliform count was determined for each fish studied. The results are presented as the mean ± EE, the statistical significance $p < 0.05$. The data obtained was processed using the Sigma plot statistical program version 12.0.

III.RESULTS AND DISCUSSION

A total of 45 bacterial strains (Table 2) corresponding mostly to *Escherichia coli* and a low number of *Enterobacter aerogenes* were isolated from the samples. Of the 45 isolates 82.22% corresponded to *E. coli* and 17.78% to *Enterobacter aerogenes* of the total isolates.

Table 2. Microorganisms isolated in each sample

	M1	M2	M3	M4	M5	Total
E. coli	7	10	7	6	7	37
Enterobacter aerogenes	2	2	1	1	2	8

The most likely number of total Coliforms per gram was similar in the 5 samples, as shown in Table 3.

Table 3. Average ± SE of the most likely number of total Coliforms g^{-1}, made to 50 specimens.

M1	M2	M3	M4	M5
1100	1100	1100	1100	500
1100	1100	1100	1100	1100
1100	1100	1100	1100	1100
1100	1100	1100	1100	1100
1100	1100	1100	1100	1100
1100	1100	1100	1100	1100
210	210	1100	1100	500
500	500	500	500	1100
1100	1100	500	500	1100
210	210	1100	1100	500
X±EE 862±123	862±123	980±80	980±80	920±91

The mean of the most likely number (MPN) of total coliforms in the different samples showed that there was no statistically significant difference ($p > 0.05$). These data show that the natural environment in which these fish live has a high load of polluting bacteria belonging to the coliform group, considering that the bacterial flora depends on the type and number of microorganisms found in the environment, most of which correspond to Gram-negative bacteria, although there are some variations in tropical climates [23].

The antibiotics evaluated are part of the arsenal of drugs used in the treatment of infections in our environment and correspond to broad-spectrum antibiotics. This can be seen in Table 4.

Table 4. Characterization of microorganisms isolated in resistant, intermediate sensitivity and susceptibility to antibiotics assessed

Isolated			
	Resistant	**Moderately resistant**	**Susceptible**
CRO	1	1	23
C	6	6	27
OX	29	0	5
AN	0	8	21
SXT	7	5	14
AM	18	1	2
CTX	6	2	9

As OX and AM are the antibiotics that resulted in the highest number of resistant bacteria, these results are consistent with those found by Al-Bahry et al., [24] and McPhearson et al., [25], who isolated bacteria from fish in contaminated sites. The widespread use of antibiotics for the treatment of human infections has widely spread multi-resistant bacteria in the environment, including water [26]. It is very likely that these microorganisms reached marine waters through wastewater discharges into the marine environment. The findings found in this study may be related to the above-mentioned fact by Kaspar et al., [27].

Of the total number of isolated microorganisms, the highest percentage showed resistance to the antibiotics evaluated in greater degree to AM and OX, while the highest susceptibility was shown against C, CFX and AN. The percentage of resistance for each antibiotic of the total isolated microorganisms is shown in Fig. 1.
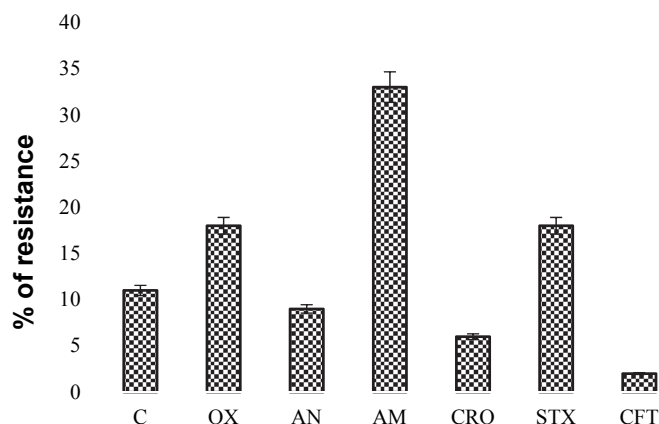
Fig. 1. Overall percentage of antibiotic resistance evaluated

With respect to the patterns of resistance to antibiotics examined, the highest percentages of resistance were shown by *E. coli* to beta-lactam antibiotics (AM and OX). Studies carried out on *Enterobacter spp* have shown the resistance of this microorganism to third generation beta-lactamic agents [23], a result similar to that shown by the isolated microorganisms in the study, which showed resistance to this type of antimicrobial agents. Table 5 shows the number of bacteria resistant to the different antibiotics evaluated in this research.

There is evidence of the generalization of resistance to multiple antibiotics, not only clinical isolates, this phenomenon has spread to bacteria isolated from the environment, among these studies is worth highlighting that carried out by Miranda and Zemelman [26]: Antibiotic resistance in fish caught from Chile's Bay of Conception showing resistance to ampicillin, tetracycline, and streptomycin, while resistance to chloramphenicol was lower. These results are compatible with those obtained in this study, which shows a high percentage of microorganisms resistant to ampicillin and the lowest resistance was shown by chloramphenicol, as shown in Table 5. In this study, isolated bacteria showed resistance to a third generation cephalosporin (Cefotaxime).

Table 5. Number of resistant strains from 1 to 4 antibiotics

Antibiotics	Number of resistant bacteria
CRO	1
C	6
OX	29
AN	0
SXT	7
AM	18
CTX	6
AM-C	2
AM-OX	8
OX-SXT	2
OX-CRO	3
OX-C	3

IV. CONCLUSION

The indiscriminate use of antibiotics is causing that the microorganisms present resistance to these, as it could be observed in the present investigation, where the bacteria isolated from the smooth ones (*Mugilincilis*) presented some degree of resistance to the antibiotic cocktail used.Oxacillin was the antibiotic to which the bacteria tested had more resistance, so it is estimated that this is the most used in the area evaluated. It was also found that the natural environment of the smooth ones contributed to increased bacterial load.

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