Serology and patterns of antimicrobial susceptibility in *Escherichia coli* isolates from pay-to-fish ponds

Sorologia e suscetibilidade antimicrobiana em isolados de **Escherichia coli** *de pesque-pagues*

Mayhara Martins Cordeiro Barbosa[1]*, Fernanda de Rezende Pinto[2], Laryssa Freitas Ribeiro[3], Cintia Sobue Lorenzon Guriz[3], Antônio Sérgio Ferraudo[3], Renato Pariz Maluta[3], Everlon Cid Rigobelo[4], Fernando Antonio Ávila[3], Luiz Augusto Amaral[3]

ABSTRACT: The occurrence of *Escherichia coli* (EPEC, EIEC and O157) in water and fish (skin, gut and muscle) in pay-to-fish ponds of the micro bay of Córrego Rico, in Jaboticabal (SP), was assessed. One hundred and fifteen strains of *E. coli* were isolated, and 49 (43%) were serogrouped as EPEC. The most common serogroups were O125, O126 and O158. Among the tested samples, 60 (52%) showed simultaneous resistance to two antimicrobials. A correspondence analysis was performed to assess possible correlations involving the site of isolation, serogroups and multi-resistance. The results of this analysis showed that the muscle was less correlated with the the other factors. However, the isolation of EPEC serogroups in this study demonstrates a risk to public health.

KEYWORDS: *Escherichia coli*; serogroup; water; fish.

RESUMO: Pesquisou-se a ocorrência de *Escherichia coli* (EPEC, EIEC, O157) em água e peixe (pele, trato digestivo e músculo) de pesque-pagues da microbacia do Córrego Rico, Jaboticabal (SP). Foram isoladas 115 cepas de *E. coli*, entre as quais 49 (43%) foram sorogrupadas como EPEC. Os sorogrupos mais frequentes foram O125, O126 e O158. Dentre as amostras testadas, 60 (52%) apresentaram resistência simultânea a dois antimicrobianos. A análise de correspondência foi realizada com o intuito de verificar as possíveis correspondências envolvendo o local de isolamento, sorogrupos e multirresistência e, com isso, pôde-se observar que o músculo apresentou menor correspondência com os demais fatores analisados. Porém, o isolamento de sorogrupos EPEC neste estudo representa risco à saúde dos consumidores.

PALAVRAS-CHAVE: *Escherichia coli*; sorogrupo; água; peixe.

[1]Instituto Federal de Educação, Ciência e Tecnologia do Ceará (IFCE) – Quixadá (CE), Brasil.
[2]Faculdade de Veterinária; Universidade Federal de Pelotas (UFPEL) – Pelotas (RS), Brazil.
[3]Faculdade de Ciências Agrárias e Veterinária; Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) – Jaboticabal (SP), Brazil.
[4]UNESP, Campus experimental de Dracena – Dracena (SP), Brazil.
*Corresponding author: mayhara@ifce.edu.br
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INTRODUCTION

Aquaculture is increasing fast in several places of the world. Because the products of aquaculture are important sources of food, it is economically important (Naylor et al., 2009). In rural Brazil, aquaculture is widely developed in environments in which domestic animals (pigs, ducks, poultry or cattle) are common. In this context, if husbandry is conducted incorrectly, animal feces can pollute the water and jeopardize human and animal health by the presence of undesirable pathogens.

The penetration and colonization of bacteria in different fish tissues and organs, such as the gastrointestinal tract, gills, muscle, kidney and bladder, have been reported in polluted aquatic environments (Pal; Dasgupta, 1992). Although *E. coli* is not an indigenous inhabitant of the gut microbiota of fish, this bacterium has been often isolated from the stomach and gut of fish (Guzmán et al., 2004).

Although most *E. coli* strains are non-pathogenic, some of them are highly pathogenic (Kuhnert, 2000). Enteropathogenic *E. coli* (EPEC) is the main cause of diarrhea among children in developing countries (Dean et al., 2005). It is generally transmitted by contaminated food and colonizes the small intestine, where it becomes attached to epithelial cells and produces typical lesions named "attaching and effacing" (A/E) (Kaper et al., 2004).

Serogroup O157 is the causative agent of a number of diseases, from mild diarrhea to severe diseases, such as bloody diarrhea (BD) and hemolytic uremic syndrome (HUS) (Nakanishi et al., 2009).

Enteroinvasive *E. coli* (EIEC) is seldom isolated from fish in developed countries, being more often isolated from fish in developing countries (PENG et al., 2009). The source of this pathogen is mainly contaminated food and water (Sankaran et al., 2009).

The objectives of this study were to determine the occurrence of some diarrheagenic *E. coli* strains (Epec, Eiec and O157) and to determine the antimicrobial susceptibility patterns of the isolates obtained from water and fish samples from pay-to-fish lakes.

MATERIAL AND METHODS

Five pay-to-fish ponds located in the state of São Paulo, Brazil, were used to collect water and fish from April 2008 to June 2008. From each pond, ten units of adult *Oreochromis niloticus* (Nile tilapia) were collected. All fish were placed in sterile bags containing peptone water in order to wash their body surface. Bags were transported in thermal boxes with ice (Apha, 2001).

The water samples were collected at five different points in all pay-to-fish ponds using sterile containers. For *E. coli* isolation, the water, muscle tissue and gastrointestinal tract were analyzed in the laboratory (Apha, 2001). After enrichment by Lauryl Tryptose Broth (Difco, Detroit, USA), the samples were streaked onto MacConkey agar (Oxoid, Hampshire, UK) and incubated at 37ºC for 24 hours. Thereafter, three typical *E. coli* colonies were biochemically identified by the IMViC tests (indole production, methyl red, Voges-Proskauer and citrate) (Koneman et al., 2001).

The *E. coli* isolates were then subjected to a slide agglutination test with sera against typical serogroups of EPEC (O26, O55, O111, O119, O114, O125, O142, O158, O86, O126, O127, O128), EIEC (O28ac, O29, O136, O144, O152, O112ac, O124, O143, O164 and O167) and O157 (Probac do Brasil, São Paulo, Brazil).

The presence of hemolytic activity was assessed using sheep blood Agar 5%. The plates were incubated at 37ºC for 24 hours.

The antimicrobial susceptibility was tested on Müller-Hinton agar by the disk diffusion technique (Bauer et al., 1966), according to the Clinical and Laboratory Standards Institute (CLSI, 2007). The tested antimicrobials were as follows: ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), erythromycin, (15 µg), streptomycin (10 µg), gentamycin (10 µg), neomycin (5 g), novobiocin (5 µg), sulfamethoxazole + trimethoprim (25 µg), tetracycline (30 µg) and trimethoprim (5 µg).

A correspondence analysis was performed with the objective of assessing the possible correspondences regarding the place of isolation, serogroups and resistance to one (R_1), two (R_2), three (R_3) and four (R_4) antimicrobials, as well as the isolates that could not be serotyped (ND). This analysis used the basic concept of the chi-square test, and the total data variation was provided by the inertia factor (Hair et al., 2005).

RESULTS AND DISCUSSION

After biochemical identification, 115 *E. coli* isolates were obtained; 19 were obtained from water, and 96 were obtained from fish. Regarding fish isolates, 26 were recovered from the skin, 65 from the gastrointestinal tract and five from the muscle.

The hemolysis test may be used as a marker of the virulence of microorganisms isolated in food animals (Costa et al., 2006). However, no isolate presented such a characteristic.

Five isolates (4%) agglutinated with O157 sera, while six (5%) were EIEC positive. Forty-nine (43%) agglutinated with EPEC sera (Nataro et al., 1998). The most frequent serogroups in water isolates were O125, O126 and O158; in skin, O125, O128 and O86 were more frequent; and in the gastrointestinal tract, O125, O126 and O55 were more common. No muscle isolates were typified.

The EPEC serogroups showed heterogeneous results; however, the serogroups O125, O126, O128, O158 and O55 were the most frequent ones. All of these serogroups can be found in human diseases (Kapper et al., 1998). However, Campos et al. (2004) noticed that the serogroups O126 and O128 did not show virulence factors in isolates from children with diarrhea. The serogroup O55, which is found in the gastrointestinal tract, is considered as one of the most important EPEC serogroups due to its isolation frequency and because it is often detected in diarrhea among children in Brazil. Previous work based on virulence properties and serotyping characteristics showed that the EPEC serogroups are heterogeneous, and although most strains are EPEC, many of them do not possess virulence characteristics (Campos et al., 2004).

The O157 pathotype was not found in the muscle (based on slide agglutination), but it was found in one isolate from the gastrointestinal tract and in two water isolates. This pathotype has been isolated from several food sources; cattle is the main reservoir of O157 (Paton; Paton, 2002). However, Orsi et al. (2007) found O157 genes in water used in thermal baths in Brazil, and Licence et al. (2001) noticed the contamination of the public water service by O157 *E. coli* in Scotland. According to Bugarel et al. (2011), while EPEC serogroups have been mostly related to cases of diarrhea among children in developing countries, O157 serogroups have been related to foodborne outbreaks in developed countries.

Regarding enteroinvasive *E. coli*, previous studies concerning the presence of this pathotype in the environment or in food are rare. However, analyses targeting its frequency in cases of diarrhea show that it is generally found at low percentages in food. Moreno et al. (2010) noticed a 1% rate of EIEC isolation among 290 cases of diarrhea in children in a public hospital in Brazil, while Nguyen et al. (2005) found EIEC in 7% of feces of children with diarrhea in Vietnam.

The antimicrobial susceptibility test observed that all strains were sensitive to chloramphenicol and enrofloxacin. However, the isolates were resistant to novobiocin. Table 1 shows the susceptibility to antimicrobials.

Ampicillin was the only antimicrobial to which 100% of water isolates were sensitive. From the 26 fish skin isolates, only neomycin was not resistant. From the isolates of the gastrointestinal tract, 100% of them were sensitive to tetracycline, trimethoprim and sulfamethoxazole + trimethoprim. In muscle isolates, only the antimicrobials erythromycin, cephalothin and gentamicin showed isolates with a resistance profile.

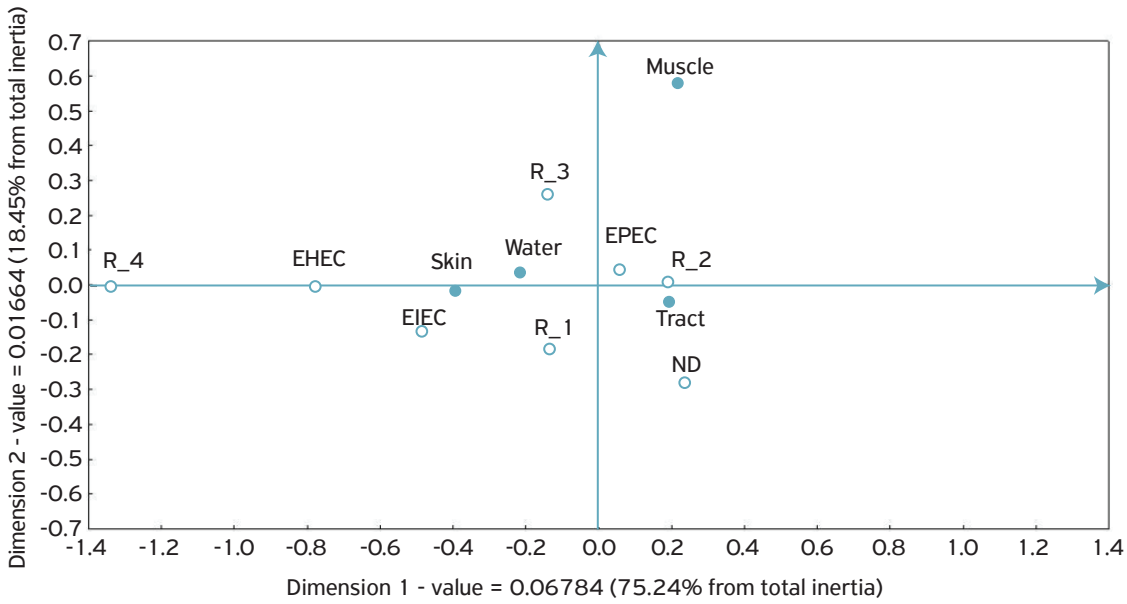
The relationship between the isolation location, multiresistance profile and serogroup was evaluated by the correspondence analysis (Fig. 1). The quality of the map is measured by the amount of inertia retained in the two considered dimensions. In this study, the retention was 93.69%, with 75.24% retained in the first dimension, and 18.45% in the second.

Muscle had the lowest correspondence compared to the remaining factors, probably because the muscle of live fish is not a natural *E. coli* habitat (Apun et al., 1999). Results indicate that the evaluation of the hygienic-sanitary quality of fish muscle does not describe the real risk of consuming fish from tanks containing pathogens, since the risk associated with contaminated fish consumption is not only associated with the bacteria that are present in the edible tissue. Infection can also occur while handling the fish; cross-contamination from other foods, surfaces and the handler's hands is likely to occur when the fish is prepared and cleaned for consumption (Strauss; Blumenthal, 1990). Based on the analysis, water, skin and the gastrointestinal tract presented a

Table 1. Susceptibility of *Escherichia coli* isolates obtained from fish samples and water from pay-fo-fish ponds to antimicrobials.

Source	S*	Antimicrobials (N)								
		AMP	GEN	NEO	STE	TET	TRI	SUT	CPL	ERI
Water	S	19	18	12	12	17	17	17	10	7
	I	0	1	7	5	0	0	0	9	0
	R	0	0	0	2	2	2	2	0	12
Skin	S	23	23	10	17	23	25	25	16	12
	I	0	2	16	6	0	0	0	6	0
	R	3	1	0	3	3	1	1	4	14
GI tract	S	64	61	13	33	65	65	65	39	19
	I	1	5	51	31	0	0	0	18	0
	R	0	0	1	1	0	0	0	8	46
Muscle	S	5	4	1	4	5	5	5	2	0
	I	0	0	4	1	0	0	0	2	0
	R	0	1	0	0	0	0	0	1	5

*Susceptibility: S - sensitive; I - intermediary; R - resistant
AMP: ampicillin; GEN: gentamicin; NEO: neomycin; STE: streptomycin; TET: tetracycline; TRI: trimethoprim; SUT: sulfamethoxazole + trimethoprim; CPL: cephalothin; ERI: erythromycin.



EHEC: Enterohemorrhagic Escherichia coli serogroup; EPEC: Enteropathogenic Escherichia coli serogroups; EIEC: enteroinvasive Escherichia coli serogroups; R_1: Isolates with resistance to one antimicrobial; R_2: Isolates with resistance to two antimicrobials; R_3: Isolates with resistance to three antimicrobials; R_4: Isolates with resistance to four antimicrobials; ND: Isolates that could not be serotyped.

Figure 1. Percentage map derived from the correspondence analysis.

higher correspondence and a higher number of isolates; thus, they are possible means for the transmission of *E. coli* serotypes that cause diarrhea.

Another important aspect to be considered is the fact that the isolates presented a high amount of multi-resistance. Enterohemorrhagic *E. coli* (EHEC) isolates showed more correspondence to resistance to four antimicrobials. According to Mora et al. (2005), multi-resistant EHEC strains from food and human beings have been isolated in several countries.

EPEC serogroups were resistant to one, two and three antimicrobials, and the EIEC serogroups showed resistance to one and three antimicrobials. Hien et al. (2007) noticed that in areas of Vietnam, where the use of wastewater is allowed in agriculture and aquaculture, one out of three EIEC strains isolated from children showed resistance to all of the analyzed antimicrobials, and EPEC strains, among other antimicrobials, were resistant to gentamicin, tetracycline, streptomycin and trimethoprim.

The use of antimicrobials in aquaculture is a risk to public health because it can spread resistance genes to microorganisms that are pathogenic to human beings, such as *Aeromonas* spp. and *E. coli*, which are frequently present (Heuer et al., 2009).

The high proportion of bacteria that are resistant mainly to erythromycin, cephalothin and streptomycin suggests that other factors, besides the use of antimicrobials in pay-to-fish lakes, can favor the persistence of resistant strains. These factors can be related to inadequate handling procedures in the environment. Antimicrobials such as erythromycin and streptomycin are used to treat and prevent diseases in aquaculture; therefore, a higher prevalence of bacteria resistant to these drugs in this activity has recently been reported (Akinbowale et al., 2006).

A high number of isolates was resistant to erythromycin and novobiocin. Similar results were obtained by Carneiro et al. (2007), when they studied the susceptibility patterns of bacteria from the *Enterobacteriaceae* family isolated from water and fish from tanks. However, Sarter et al. (2007) found a higher resistance to ampicillin and chloramphenicol in *E. coli* isolated from farmed fish.

Despite the fact that the gastrointestinal tract showed a higher number of isolates, the frequency of multi-resistance was lower compared to the remaining isolates in the study. There was also higher correspondence between the gastrointestinal tract and resistance to two antimicrobials. Lima et al. (2006) made a similar observation and suggested that tge gastrointestinal tracts of fish may not be the main source of multi-resistant bacteria. However, even with a lower number in *E. coli* isolated from the gastrointestinal tracts of fish, the exchange of resistance factors between these bacteria and those from fish microbiota and the aquatic environment may be possible.

Normally, aquatic bacteria are not different from others when being exposed to antimicrobials, in that they are able to transfer resistance genes to other bacteria. Perhaps because the infections in fish and human beings are caused by bacteria that belong to the same genera, the likelihood of resistance to antimicrobials of the spreading of bacteria from aquaculture to human beings may increase. Furthermore, the consumption of contaminated fresh meat induces cross-contamination, as well as the consumption of bacteria resistant to antimicrobials. Treatment options may become limited if multi-resistant and resistant bacterial

strains are transferred from contaminated food to human beings (Hammerum; Heuer, 2009).

CONCLUSIONS

A number of *E. coli* related to enteric illness has been isolated from water and from fish from pay-to-fish ponds. Contaminated fish, when handled, can play a role in the transmission of these agents to consumers, as well as contaminate food and surfaces. The correspondence analysis has shown that *E. coli* isolates from the skin, water and gastrointestinal tract have correspondence. Furthermore, the use of antimicrobials increases the risk of contamination due to acquired multi-resistance. All isolates showed resistance to at least one antimicrobial.

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