

Original Research Article

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Relationship between the Phylogenetic Group of *Escherichia coli* Strains Isolated in Water and Fish in Fresco Lagoon (Côte d'Ivoire)

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ABSTRACT

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The aim of this study was to know the quality of water and some of the fish from Fresco lagoon, a RAMSAR site. In total, 376 samples including 216 water samples and 160 fish (viscera and gills) were collected in six sites and lagoon respectively. They analysed by the standard microbiological test for *Escherichia coli* isolation. Identification and phylogenetic affiliation of *E. coli* were performed by PCR. In water, 338 strains of *E. coli* were isolated. From fish, 86 (53.75%) *E. coli* were isolated from the viscera and 74 (46.25%) from the gills. In water and fish, the phylogenetic group A was the most frequent (69.82% and 81.25%, respectively), followed by the group B1 (23.67% and 13.75%, respectively). The group B2 was rare in water (4.44%) and fish (5%) while D (2.07% in water) was absent in fish. Strains of groups B2 and D are usually more pathogenic than A and B1 and they are frequently responsible for extra-intestinal infections. Our findings indicate that Fresco lagoon could represent a potential reservoir of extra-intestinal infections for the riverine populations and consumers.

Introduction

Fresco lagoon, like many African coastal lagoons, is under consistent and sometimes severe pressure from diverse forms of human activities emanating from the surrounding city and villages. The indiscriminate dumping of untreated wastes into this aquatic environment could bring about physical, chemical and biological deteriorations of the water body. This will no doubt endanger the resident aquatic

organisms as well as impair the beneficial uses of the water. However, a paucity of information exists on the extent of biological pollution of the Fresco lagoon and hence its public health implication. In a recent study, we isolated fourteen biotypes of *E. coli* from fishes and water samples from Fresco lagoon (Kouadio *et al.*, 2011).

Escherichia coli is a normal inhabitant of the lower intestinal tract of warm-blooded

animals and humans. However, in recent years, this species was found in some cold-blooded animals such as fish (Hansen *et al.*, 2008; Yagoub, 2009; Soliman *et al.*, 2010; Sujatha *et al.*, 2011). Phylogenetic studies show that there are four main phylogenetic groups of *E. coli*, designated A, B1, B2, and D (Herzer *et al.*, 1990; Clermont *et al.*, 2000). According to Lecointre *et al.*, (1998), groups A and B1 are sister groups whereas group B2 is included in an ancestral branch. These phylo-groups apparently differ in some characteristics, such as their ability to use different sugar sources, their antibiotic-resistance profiles and their growth rate. Walk *et al.*, (2007) demonstrated that the majority of the *E. coli* strains that are able to persist in the environment belong to group B1. *E. coli* strains are usually referred to as commensal, intestinal or extra-intestinal pathogenic (Russo and Johnson, 2000). Extra-intestinal pathogenic strains belong to groups B2 and D (Picard *et al.*, 1999; Johnson and Stell, 2000), commensal strains to groups A and B1 (Johnson *et al.*, 2001). The pathogenic strains have been associated with several diseases including diarrhea, urinary tract infections and meningitis (Russo and Johnson, 2003; Unno *et al.*, 2009).

The aim of this study was to know the quality of water and some of the fish from Fresco lagoon, a RAMSAR site.

Materials and Methods

Water and fish sampling

A total of 216 water samples were collected from Fresco lagoon. The sampling sites (figure 1) were purposely selected from Fresco city and its surrounding areas based on the direct influence of sewage and two rivers Niouniourou and Bolo. Water samples (250 ml) were collected at each site, 20 cm

below the surface of the lagoon using sterile glass bottles, stored on ice and transported to the laboratory. All the samples were collected monthly from January 2008 to June 2009. Moreover, 160 fish samples (*Tylochromis jentengi*, *Sardinella maderensis*, *Sarotherodon melanotheron* and *Chrysichthys nigrodigitatus*) were harvested in the lagoon during the same period. Immediately after collection, samples were chilled in an ice cooler box at 4 °C and transported to the laboratory of the Oceanology Research Centre (CRO) for analysis within 24 h after sampling.

***E. coli* strains isolation and identification**

A volume of 100 ml of water were filtered using membrane filter technique (U.S. EPA; 2002) and placed directly on EMB agar medium (Merck, France). For fish samples, the skin was washed with 70% ethanol before taking the gills and viscera. Both, 10 g of gills and viscera were taken aseptically and were suspended each one in 90 ml in sterile peptone water solution in glass bottles and incubated at 37°C during 24 hours. Aliquots of 0.1 ml of the suspension were inoculated onto EMB agar medium. The culture plates were then incubated at 37 °C for 24 h.

Five typical colonies of *E. coli* purple with metallic sheen per plate, were purified by inoculating onto two duplicates of Plate Count Agar (PCA, Merck, Germany), subsequent growth for 18-24 hours at 37°C and subjected to cytochrome oxidase and catalase production. Biochemical identification was done using the API 20E kit (Biomerieux SA, France) according to the manufacturer's instruction.

Stock cultures were stored in Brain Heart Infusion broth (BHI) (Difco, Michigan, USA) with 20% glycerin at -80°C until use.

Extraction of bacterial DNA

Genomic DNA of typical *E. coli* isolates were grown in Luria-Bertani (LB) broth at 37 °C overnight. Bacteria from 1.5 ml of growth medium were pelleted by centrifugation at 1200 g for 10 min. The bacterial pellet was suspended in 200 µl of sterile ultrapure water and was boiled at 100 °C for 10 min. The mixture was centrifuged again at 1200 g for 10 min. The supernatant containing the DNA was stored at -20°C and used as template in the PCR assays.

Phylogenetic group determination

The phylogenetic group of each isolated strain was determined by the triplex PCR as previously reported by Clermont (Clermont *et al.*, 2000). The amplified genes were *chuA*, *yjaA* genes and DNA fragment TSPE4.C2. Amplification was performed in a PCR thermocycler Biometra Uno II, (Biotron 1998, Germany) according to Clermont *et al.*, (2000) program.

The PCR products were analyzed in 1.8% agarose gel prepared in 0.5X TBE buffer (Tris Borate EDTA, Fermentas) containing ethidium bromide (0.2 µg/ml). Electrophoresis was carried out at 100 volts for 40 min. Finally, the gel was photographed using UV light (Uvitec camera, United Kingdom). A 1 kb DNA molecular size marker (BioLabs, France) was included in all gels to allow standardization and sizing.

Phylogenetic groups were determined using the dichotomous tree described by Clermont (Clermont *et al.*, 2000).

Statistical Analysis

The software ADE4 was used to study the link between phylogenetic groups or

subgroups and their host. A Factorial Correspondence Analysis (AFC) was performed using the phylogenetic groups and subgroups distribution. Sites and fish classification were based on these distributions.

Results and Discussion

Isolation rates of *E. coli* in fish samples

One hundred and sixty (160) strains of *E. coli* were isolated from fish samples. The identification of *E. coli* isolated in different fish species indicated that *Sarotherodon* (72.5%) seemed to be more contaminated than other fish species followed by *Tylochromis* (47.5%), *Sardinella* (45%) and *Chrysichthys* (35%). The distribution of *E. coli* within different parts of fish collected from the lagoon showed that viscera was most contaminated (53.75%) than gills (46.25%). *Tylochromis* and *Sarotherodon* showed high percentage of *E. coli* isolates from gills 52.63% and 68.97% respectively. However, *Sardinella* and *Chrysichthys* showed high percentage in the viscera 83.33% and 71.43% respectively (Figure 2).

Phylogenetic group of strains isolated from water and fishes

Dichotomous decision tree to determine the phylogenetic group of *E. coli* strain revealed the presence of groups A, B1, B2 and D in water strains. Of 338 strains of *E. coli* isolated from Fresco lagoon water, strains of group A were the most frequent (69.82%), followed by strains of group B1 (23.67%). Strains of groups B2 and D were rare (4.44% and 2.07% respectively) (Table 1). As opposed to groups B2 and D, strains of groups A and B1 were isolated at all the sampling sites. The highest percentages of strains from group A were found at sites 1 and 6 (17.75% and 17.16% respectively).

Our results also showed that only site 4 exhibited the four phylogenetic groups. Sites 1, 3 and 6 on one hand and the sites 2 and 5 on the other hand have the same phylogenetic profiles.

Of the 160 strains recovered from fish species, only three phylogenetic groups A, B1 and B2 divided in 4 subgroups A0, A1, B1 and B23 were found (Tables 2). A0 and A1 are including in group A, subgroup B1 in group B1 and subgroup B23 in group B2. The majority of strains isolated were from group A (81.25%). The groups B1 and B2 respectively totalized 13.75% and 5% of all analyzed strains. Phylogenetic distribution of *E. coli* strains according to the four fish species studied showed that the phylogenetic group A is present in all species. *Sarotherodon* and *Sardinella* are the only species to have the groups A, B1 and B2 in viscera (figure 3). The phylogenetic group B1 is identified in all species except *Chrysichthys* while B2 is present only in viscera of *Sarotherodon* and *Sardinella*.

Relationship between phylogenetics groups and their host

To study the link between phylogenetic groups or subgroups and their host, a Factorial Correspondence Analysis (AFC) was performed using their (the phylogenetic groups and subgroups) distribution (Tables 1 and 2). The bidimensional representation of phylogenetics group distribution in each site is shown in Figure 4. This representation can explain 95.08% of the total inertia. The horizontal axis represents 58.99% of the total inertia. According to this analysis, the group distribution revealed that sites 1, 2, 3 and 6 were similar and influenced by *E. coli* phylogenetic groups A, B1 and B2. Site 4 was particularly influenced by *E. coli* phylogenetic group D. Site 5 is under the influence by *E. coli* phylogenetic group B1.

Sites 1, 2, 3 and 6 are linked and represented one group and sites 4 and 5 the second ones.

Correspondence analysis between fish according to phylogenetic subgroup revealed that *Sarotherodon melanotheron* and *Sardinella maderensis* were influenced by *E. coli* phylogenetic subgroups B1 and B23. *Tylochromis jenteki* and *Chrysichthys nigrodigitatus* are under the influence of strains belonging to the subgroup A1 (Figure 5). The similarity among fishes revealed that fishes were split up in three (height 0.2), *Chrysichthys* group, *Tylochromis* group and *Sardinella* with *Sarotherodon* in the same group.

Escherichia coli is the most important microbiological parameters taken into account in controlling the quality of bathing waters and its presence is enough to confirm that it is indeed a pollution from fecal contamination (Joly and Reynaud, 2003). For surface water used as a primary contact recreation, the fecal coliform counts shall not exceed 100 CFU/100 ml of water analyzed (WHO, 1998). The survey of the *E. coli* along the Fresco lagoon revealed that this lagoon is not polluted as the bacterial counts were below these guidelines for minimizing the health risks associated with water contact activities. However, the pollution can increase over the years due to the population behaviors. As the population of Fresco grows the waste generation also increases. Many illegal sewers, especially from markets and septic tank effluent will continue to directly enter into the lagoon and increase the pollution.

Bacteria form a link between primary producers and consumers; it would therefore appear that pollution affects the aquatic food chain. The use of *E. coli* as a sanitary indicator for fish samples has been first reported in the 1930s (Costa, 2013). Fecal

coliforms in fish are influenced by fish feeding habits and fish can harbor fecal coliforms up to 14 days after being exposed to contaminated water (Hansen *et al.*, 2008). The analyses of *E. coli* presence in different fish species indicated that *Sarotherodon* seemed to be more contaminated, followed by *Tylochromis*, *Sardinella* and *Chrysichthys*. *Sarotherodon*, *Chrysichthys* and *Tylochromis* are bottom feeder while *Sardinella* is filter feeder. The presence of *E. coli* in our bottom feeder could be explained by the fact that these fishes live and feed in the mud (sediment) where the presence of *E. coli* is generally more abundant than in water (Desmarais *et al.*, 2002; Ishii *et al.*, 2007). Sediment materials probably provide to bacteria some protection against light-induced inactivation.

Considering these facts, it appears less likely that pelagic fish (*Sardinella*) will ingest or be inoculated by as many *E. coli* as benthic fish (Hansen *et al.*, 2008). Analyses of the fish gills and viscera showed that the rate of *E. coli* in *Sardinella* and *Chrysichthys* is higher in viscera than in gills. These results agreed with those of Lee and Marks (2009) who indicated that *E. coli* is a bacterium that commonly lives in the intestines of people, animals and fish. Hansen *et al.*, (2008) also reported that *E. coli* was firstly discovered in intestine of wild fish. Fish may also absorb bacteria from its feeding and surrounding water, these bacteria accumulate in various organs in significant proportions.

Since contamination from *E. coli* represents a risk for human health, we decided to gather more information about *E. coli* strains isolated from water and fish samples by assigning them to the phylogenetic groups A, B1, B2 and D using the PCR based technique described by Clermont *et al.* (2000). As expected, the four phylogenetic groups were found within water isolates

strains. Strains belonging to groups A and B1 are the majority with more than 75% strains distributed across all sites. Duriez *et al.*, (2001) found that in 168 non-epidemiological related isolates from three geographically distinct human populations, strains from phylogenetic groups A and B1 were the most common; strains of the group B2 were rare. *E. coli* strains are usually referred to as commensal, intestinal or extraintestinal pathogenic (Russo and Johnson, 2000). Extraintestinal pathogenic strains belong to groups B2 and D (Johnson and Stell, 2000; Clermont *et al.*, 2000), the commensal strains to groups A and B1 (Johnson *et al.*, 2001). Our study demonstrated that the commensal are the most abundant strains in the water of Fresco lagoon. This may confirm the fact that the lagoon is the receptacle of humans and animals' feces. *E. coli* isolated from lagoon water showed the 4 phylogenetic groups not only among isolates from different sites but also among those of the same site. The relationships between site according to the distribution of phylogenetic groups revealed that sites were classed in 2 groups. The group composed by sites 4 and 5 could be under same multiple sources of fecal contamination. They received river inputs (Niouniourou and Bolo), wastewaters, sediment, and uncontrolled urban discharges.

E. coli isolated from fish showed 3 phylogenetic groups A, B1 and B2 not only among isolates from different fish species but also among those of the same fish species. Strains belonging to groups D were not found in fish. The relationships between fishes according to the distribution of phylogenetic groups revealed that fishes were classed in 3 groups where *Sardinella* and *Sarotherodon* constituted the same group.

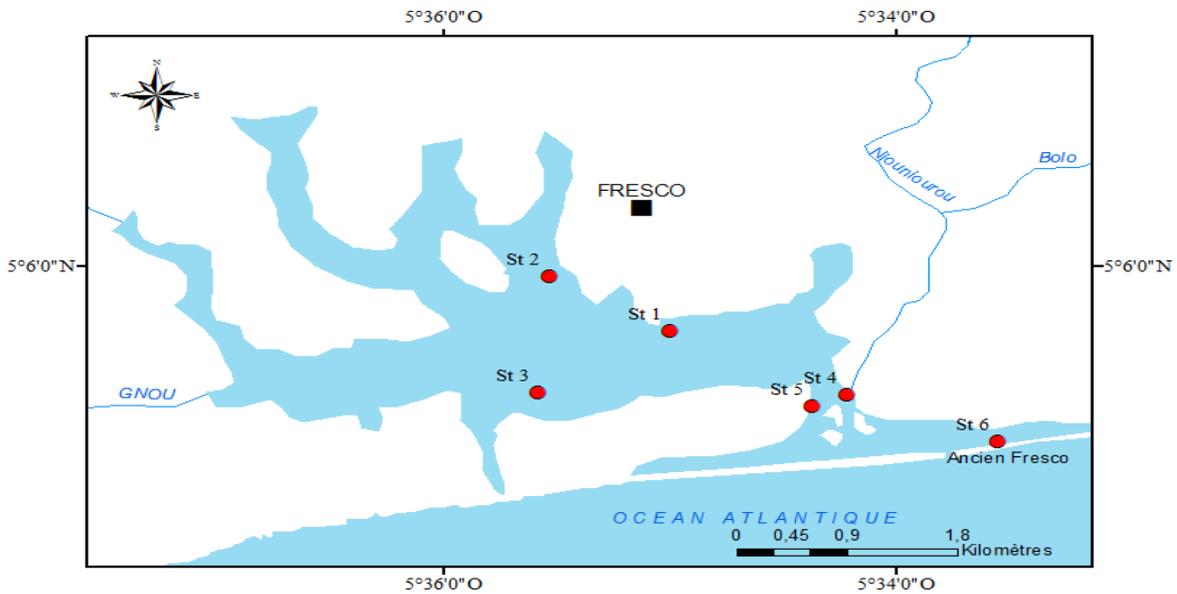
Table.1 Distribution of *E. coli* strains from water of Fresco lagoon among the four phylogenetic groups according to sites (%)

Phylogenetic groups	Sites						Total
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	
A	17.75	9.76	7.40	9.17	8.58	17.16	69.82
B1	4.73	5.03	1.18	1.78	4.73	6.21	23.67
B2	1.18	00	1.18	0.59	00	1.48	4.44
D	00	0.89	00	0.59	0.59	00	2.07

Table.2 Distribution of the *E. coli* phylogenetic subgroups among fish analyzed

Subgroups phylogenetic	Fish species				Total
	<i>Tylochromis</i>	<i>Chrysichthys</i>	<i>Sarotherodon</i>	<i>Sardinella</i>	
A1	34	28	39	22	123
A0	2	0	3	2	7
B1	2	0	12	8	22
B23	0	0	4	4	8
Total	38	28	58	36	160

Fig.1 Map of Fresco Lagoon showing sampling sites.



St : site

Fig.2 Proportion of *E. coli* isolated according to the different parts of fish species analyzed

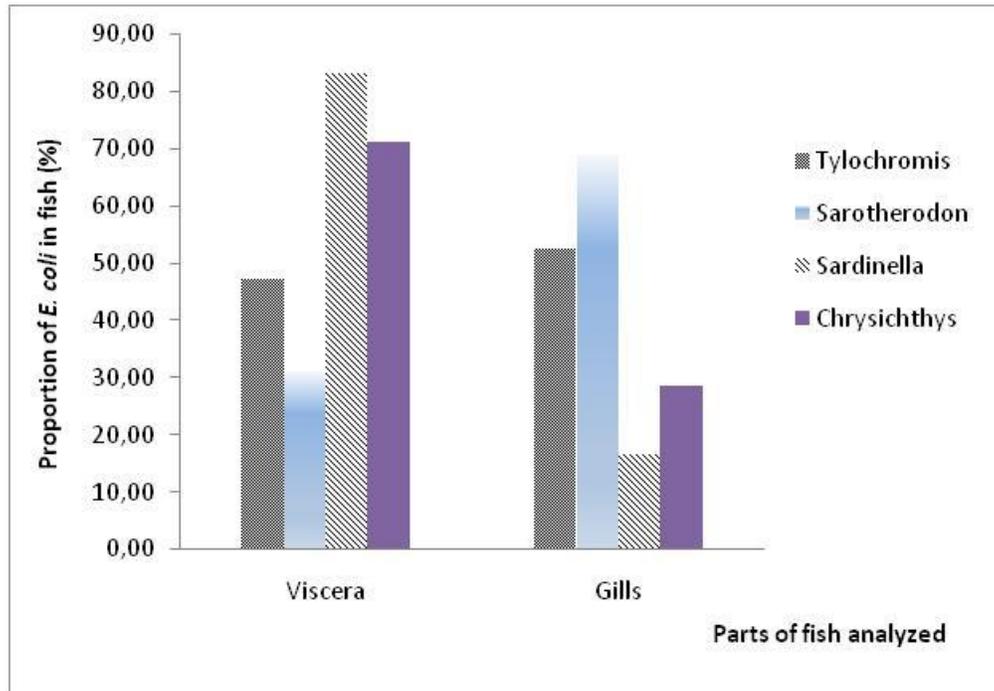


Fig.3 Proportion of *E. coli* phylogenetic groups according to parts of fish species (%).

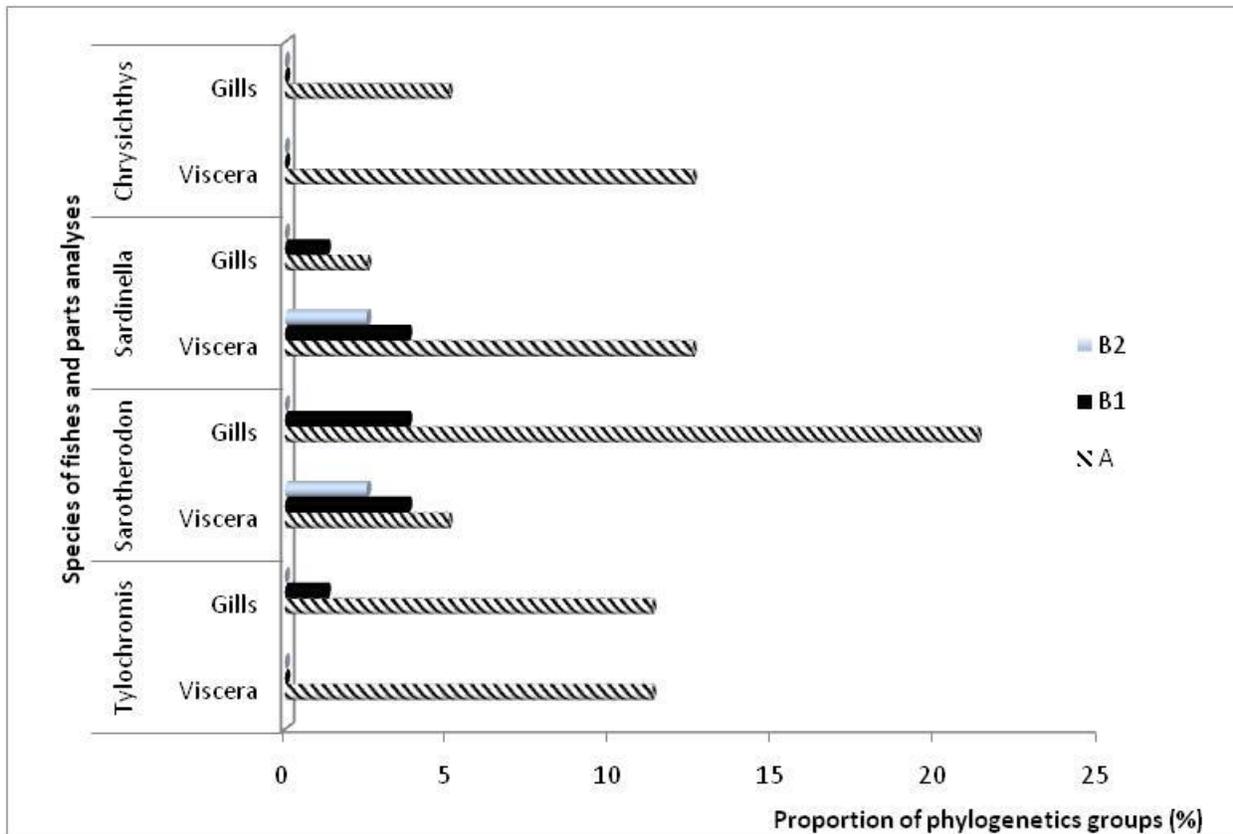


Fig.4 Correspondence analysis of sites using phylogenetic groups repartition.
 ST1: site 1, ST2: site 2, ST3: site 3, ST4: site 4, ST5: site 5, ST6: site 6

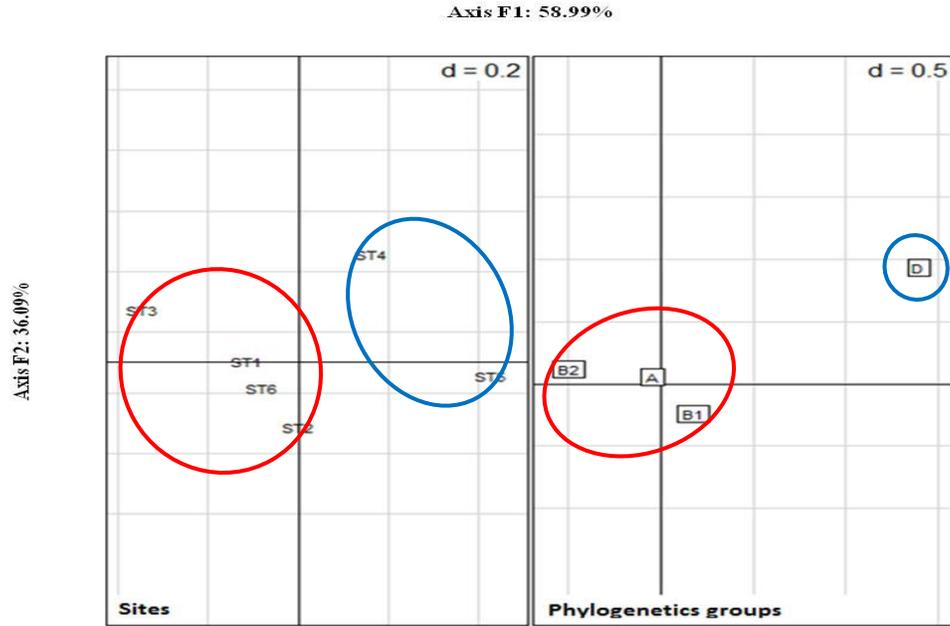
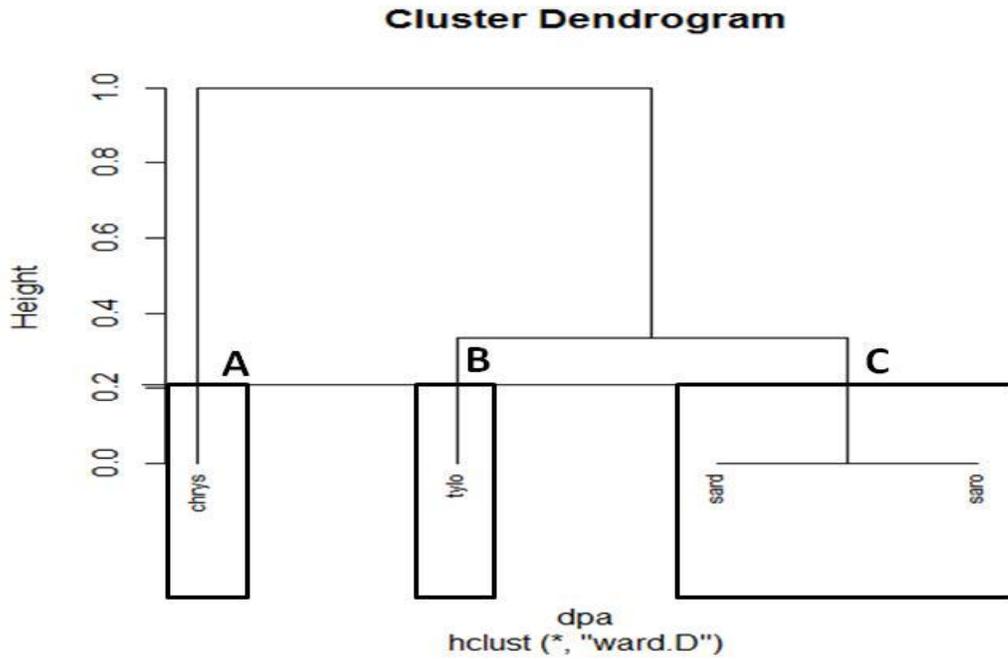


Fig.4 Dendrogram for clustering fishes according to *E. coli* phylogenetic subgroups repartition
 Chrys: *Chrysichthys*; Tylo: *Tylochromis*; Sard: *Sardinella*; Saro: *Sarotherodon*
 A: Fish species contaminated by *E. coli* phylogenetic subgroup A1,
 B: Fish species contaminated by *E. coli* phylogenetic subgroup A1,
 C: Fish species contaminated by *E. coli* phylogenetic subgroup B1 and B2



According to Hume (1999), host habitat, diet, "typical" body temperature and gut morphology are all factors that influence the distribution of *E. coli* phylogenetic groups among host individuals.

Strains belonging to groups B2 and D are less represented in water and fish samples. These results are in agreement with those of Walk *et al.*, (2007). Picard *et al.*, (1987) found that B2 strains accounted for only 9% of examined commensal human strains. *E. coli* strains from group B2 are highly pathogenic and frequently responsible for extraintestinal infections (Lecointre *et al.*, 1998; Duriez *et al.*, 2001; Orsi *et al.*, 2007) and for urinary tract infections in humans (Zhang *et al.*, 2002). Extraintestinal pathogenic *E. coli* can be found in group D (Picard *et al.*, 1999; Orsi *et al.*, 2007) and according to Clermont *et al.*, (2000), *E. coli* O157:H7 could belong to this group. The smaller percentage of phylogenetic groups B2 and D found in our study confirms the hypothesis that these strains derive from commensal strains by the acquisition of chromosomal or extra-chromosomal virulence operons (Finlay and Falkow, 1997; Ochman *et al.*, 2000). It is also reported that "black hole" genomic deletions that enhance pathogenicity or random functional point mutations that are adaptive for pathogenic environments (Sokurenko *et al.*, 1998) may represent additional strategies of genome plasticity by which a commensal strain may become virulent. The presence of groups B2 and D in the water might suggest that Fresco lagoon can present a risk of extra-intestinal pathogenic *E. coli* for inhabitants who use this water for recreational activities. Strains of group B2 were also found in fishes. This finding raises serious worries in the sense that badly gutted and cleaned fish that have undergone little or no cooking can be a source of contamination.

In conclusion, the results obtained in this work indicate that the Fresco lagoon is not polluted according to the WHO guideline. Commensal and extra-intestinal pathogenic *E. coli* were isolated from water and fish samples. Fresco lagoon could represent a potential reservoir of extra-intestinal infections for the riverine populations and consumers. Therefore, riverine population must be educated in order to minimize the fecal contamination.

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