# Pathogenic, phylogenetic, and antibiotic-resistant profile of Escherichia coli in aquatic environments

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# Background

Methodology

Escherichia coli is a commensal bacterium that inhabits the intestine of humans and other warm-blood animals<sup>1</sup>, and thus it is routinely used as a microbiological indicator of fecal contamination<sup>2</sup> in the aquatic environment. Several *E. coli* strains can be pathogenic, causing both extra-intestinal and intestinal diseases in humans, being therefore of public health concern<sup>3</sup>.

The aim of this study was to investigate the origin and the pathogenic profile of the fecal indicator bacteria (FIB) Escherichia coli in temperate Atlantic recreational waters, and to explore eventual potential implications for public health.

#### Characterization of E. coli isolates according to phylogenetic groups Membrane Fecal Coliform (mFC) Detection and isolation were n = 362 n = 272 performed using selective and differential culture media ---------Chromocult Coliform Agar (CHR) E. coli presumptive isolates were Characterization of E. coli isolates according confirmed by PCR to pathotypes (virulence profiles) **Sample Collection** Results Assessment of antibiotic susceptibility **Aquatic Environment** Affluent Resistant < 3 classes Resistant ≥ 3 < 6 classes ■ Resistant ≥ 6 classes Coastal Multidrug resistance Resistance Effluent Transitional + Wastewater 28% 66% Resistance Resistant Susceptible 50% 80% 30% 60% 70% Percentage of isolates ETEC n = 4 **EPEC** Commensal n = 3 n = 26 Commensal **EHEC** n = 36 n = 2 EPEC/EAEC ETEC/EHEC ETEC EHEC/ EHEC n = 2 **EHEC** Commensal **EAEC** EAEC n = 4n = 19 ETEC n = 3**EPEC** n = 6 **EPEC EHEC** ETEC/ n = 3 n=2EPEC, n=1 Phylogenetic subgroups $A_0 A_1 B1 B2_2 B2_3 D_1 D_2$ E. coli (ETEC).

Hierarchical clustering and heatmap of the antibiotic resistance profile of *E. coli* isolates according to the aquatic environmental source. Isolates with 100% of similarity were clustered together. Thicker vertical lines divided the 9 different antibiotic classes used. From left to right: Cephalosporins – Ceftazidime 30 μg, Cefotaxime 5 μg, Cefoxitin 30 μg, Cephalothin 30 μg; Aminoglycosides – Amikacin 30 μg, Kanamycin 30 μg, Gentamicin 10 μg, Tobramycin 10 μg; Tetracyclines – Tetracycline 30 μg; Macrolides - Erythromycin 15 μg; Fluoroquinolones - Nalidixic acid 30μg, Ciprofloxacin 5 μg; Miscellaneous agents - Streptomycin 10 μg, Nitrofurantoin 300 μg, Trimethoprim - sulfamethoxazole 25 μg, Chloramphenicol 30 μg, Rifampicin 5 μg; Monobactams - Aztreonam 30 μg; Carbapenems - Imipenem 10 μg, Ertapenem 10 μg and Penicillins – Ampicillin 10 μg, Amoxicillin – clavulanic acid 30 μg.

Percentage of *E. coli* isolates resistant to different antibiotic classes (n = 272). Tree map showing the number of *E. coli* isolates with virulence genes (pathotypes), and multidrug resistance grouped according to phylogenetic subgroup (n = 195). Enteroaggregative E. coli (EAEC), Enterohaemorrhagic E. coli (EHEC), Enteroinvasive E. coli (EIEC), Enteropathogenic E. coli (EPEC) and Enterotoxigenic

- Higher frequency of non-human mammals and birds origin subgroups (B1 and D<sub>1</sub>), compared to the predominantly human associated subgroups (B2₂ and B2₃).
- 72% of the isolates showed resistance at least 3 different antibiotic classes, therefore considered multidrug resistant.
- Among Diarrheagenic *E. coli* isolates 75%, exhibited multidrug resistance.
- Phylogenetic subgroup A1 (non-humam mamals and humans), and group B1 showed higher virulence and multidrug resistance rates.

## Conclusions

- The outcomes highlighted the circulation of pathogenic and antibiotic-resistant *E. coli* in recreational waters. Moreover, non-human mammals and birds appear to play a key role in fecal contamination rather than sewage.
- This study emphasizes the importance of additional monitoring parameters to improve public health risk management to guarantee the safety of recreational water users.



### References

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90%

Commensal

n = 13

**EHEC** 

n = 8

100%

ETEC

EIEC EPEC EPEC,

Commensal

n = 10

Commensal

n = 14

EIEC

n = 5

ETEC

n = 2

EHEC EIEC

n = 1 | n = 1

ETEC

n = 4

Commensal

n = 3

EIEC EPEC

EHEC

n = 1

