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Potential enterovirulence and antimicrobial resistance in *Escherichia coli* isolates from aquatic environments in Rio de Janeiro, Brazil ☆

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HIGHLIGHTS

- *E. coli* isolates were recovered from aquatic environments in Rio de Janeiro, Brazil.
- Specific gene combinations characterized ETEC, ATEC and STEC pathotypes.
- Phylotyping categorized *E. coli* isolates in A, D and B2 phylogroups.
- A diversity of antimicrobial resistance patterns was detected and defined multidrug-resistant (MDR) and possible XDR phenotypes.
- These findings alert to the high risk to ecosystem and human health.

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ABSTRACT

Escherichia coli contamination in aquatic ecosystems has emerged as a relevant concern of public health impact, especially in developing areas. In this study, *E. coli* isolates were recovered from residential, industrial, agricultural, hospital wastewaters and recreational waters and, further characterized according to diarrheagenic potential, phylotyping and antimicrobial resistance phenotype. Among the total 178 *E. coli* isolates, antimicrobial resistance was detected in 37% to at least one of the 11 antimicrobials tested. The highest percentage of resistant *E. coli* was recovered from agricultural wastewaters (57.7%) followed by recreational waters (56.4%), hospital (34.5%), residential (22.7%) and industrial wastewaters (22.2%). Twenty-three resistance profiles (I–XXIII) were detected and 17 isolates exhibited the MDR phenotype. 11.2% of the total *E. coli* isolates carried diarrheagenic markers: *astA* (7.3%, 13/178), stx_1 (2.8%, 05/178), *escV* (2.2%, 04/178) and *estIa* (0.6%, 01/178). All isolates harbored the *uidA* gene. *E. coli* isolates were mostly found in phylogenetic groups A (91.6%, 163/178) followed by groups D (5%, 09/178) and B2 (3.4%, 06/178). Specific gene combinations characterized *E. coli* pathotypes as ETEC (01/20), ATEC (04/20) and STEC (05/20) which belonged to A (75%, 15/20), D (15%, 03/20) and B2 (10%, 02/20) phylogroups. Our results revealed the widespread distribution of *E. coli* in aquatic systems in Rio de Janeiro. The circulation of pathogenic *E. coli* and antimicrobial resistance within bacterial population represents high risk to ecosystem and human health and highlights epidemiological surveillance and sanitary improvement.

1. Introduction

Sewage contamination of aquatic systems poses a definite risk to human health via waterborne pathogens, being responsible for a wide spectrum of disorders when water is used for drinking, recreational activities, shellfish harvesting or irrigation (Hamelin et al., 2006; Mokracka et al., 2011; Akter et al., 2013; Harwood et al., 2013; Lanata et al., 2013; Maal-Bared et al., 2013). The detection of waterborne pathogens is difficult, expensive, time consuming and complex due to the diversity of pathogens that are known to be present in sewage (Harwood et al., 2013). A great number of microorganisms has been identified; however, *Escherichia coli* is recognized as a relevant bacterial contaminant in surface water of aquatic environments from diverse geographic areas (Hamelin et al., 2006, 2007; Sabaté et al., 2008; Cabral, 2010; Mokracka et al., 2011; Harwood et al., 2013). *E. coli* is a bacteria member of intestinal tract of humans and other warm-blooded animals and it is brought into aquatic contamination mainly through untreated wastewater release (Ishii and Sadowsky, 2008; Torres et al., 2010; Williams et al., 2010; Harwood et al., 2013). *E. coli* is a health risk in some tropical and subtropical areas and is one of the leading causes of diseases and death in humans and animals (Williams et al., 2010; Lanata et al.,

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2013). In Brazil and in other developing countries, this bacterial species is an important cause of acute and persistent diarrheal diseases especially prevalent in infants (Regua-Mangia et al., 2004a,b, 2009a; Guth et al., 2010; Torres et al., 2010; Williams et al., 2010).

The majority of *E. coli* strains are commensal; however, some lineages have acquired specific virulence attributes that allow them to cause a wide spectrum of clinical manifestations such as diarrhea, urinary tract infection, meningitis, and septicemia (Torres et al., 2010; Williams et al., 2010). *E. coli* strains associated with diarrheal illness are categorized into distinct pathotypes or pathovars according to their specific virulence attributes (Torres et al., 2010; Williams et al., 2010). Currently, six classes of diarrheagenic *E. coli* are recognized: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC). Atypical variants have been described within these diarrheagenic categories (Kaper et al., 2004; Torres et al., 2010; Williams et al., 2010). Although the prevalence of *E. coli* in surface waters can differ greatly between locations and environments, relatively little is known about the distribution of *E. coli* pathotypes in aquatic ecosystems (Hamelin et al., 2007; Akter et al., 2013; Sidhu et al., 2013).

Phylogenetic analysis has shown that *E. coli* strains fall into four main phylogenetic groups (A, B1, B2, and D) that differ according to metabolic properties, ecological niches, life-history characteristics and propensity to cause diseases (Clermont et al., 2000). The assignment of *E. coli* strains to one of these groups is the basis of phylogenetic studies within species. Phylogenetic groups comprise pathogenic strains and commensal isolates recovered from a wide variety of hosts, environments, geographical origins and clinical manifestations. Extraintestinal *E. coli* strains are derived from group B2 and to a lesser extent from D, commensal from A and B1, and diarrheagenic strains from groups A, B1 and D (Hamelin et al., 2007; Gordon et al., 2008; Regua-Mangia et al., 2009b). Phylogenetic studies with *E. coli* have been carried out worldwide with bacterial isolates from clinical sources; however, a few studies have determined the proportion of pathogenic or potentially pathogenic *E. coli* in the environment. The circulation of *E. coli* strains carrying diarrheagenic and extra-intestinal genetic markers has been detected in aquatic ecosystems from distinct geographic areas exhibiting particular pathogenic profiles (Hamelin et al., 2006; Akter et al., 2013; Sidhu et al., 2013). The predominance of phylogroups in aquatic environments seems to vary according to geographical location and site of contamination (Hamelin et al., 2007; Sabaté et al., 2008; Mokracka et al., 2011).

Virulence and antimicrobial resistance properties are mainly encoded on plasmids, bacteriophages, or pathogenicity islands (Djordjevic et al., 2013). These genetic elements contribute to the rapid evolution of *E. coli* strains and to the creation of new pathogenic variants since they are frequently subject to genetic rearrangements (Djordjevic et al., 2013; Harwood et al., 2013). Assessment of virulence and antimicrobial resistance in waterborne *E. coli* isolates has revealed a high diversity within bacterial populations (Hamelin et al., 2006, 2007; Sabaté et al., 2008; Servais and Passerat, 2009; Harwood et al., 2013; Mokracka et al., 2011). These findings seem to reflect the methodology used, geographic region and the environment contamination source that may contain chemicals, pharmaceutical substances as well as pathogenic microorganisms (Hamelin et al., 2006, 2007; Servais and Passerat, 2009; Lienert et al., 2011; Mokracka et al., 2011; Amaya et al., 2012; Djordjevic et al., 2013; Maal-Bared et al., 2013). The release of (multi)resistant *E. coli* strains harboring enterovirulence traits into the environment may create potential threat and become a public health risk.

As the economic and clinical impact of *E. coli* infections are considerable, monitoring of pathogenic strains in environmental reservoirs has become a serious concern worldwide, especially in developing countries. In order to better understand the current widespread of these pathogens in aquatic environments in Rio de Janeiro, Brazil, we investigated antibiotic resistance patterns, phylogenetic groups, diarrheagenic markers and the most common pathotypes of *E. coli* isolates found in surface water of urban and rural aquatic ecosystems.

2. Materials and methods

2.1. Sampling and E. coli isolates

From December 2009 to January 2011 surface water samples were collected from different aquatic environments in Rio de Janeiro chosen to represent residential, industrial, agricultural, hospital wastewaters and recreational waters. The sampling sites were randomly distributed throughout these environments. No more than one sample was taken at each site on each occasion. Sampling, transport and storage as well as bacteriological procedures followed previous recommendations (Rebello et al., 2013). Briefly, an aliquot of 60 mL was aspirated from the upper layer of the water column and filtered on cellulose acetate membrane (Millipore). The membrane containing the retained bacterial cells was incubated overnight in tryptic soy broth (TSB — Difco) at 37 °C. After the period of bacterial growth, serial dilutions were prepared in 0.9% NaCl (w/v) and inoculated onto Eosin Methylene Blue agar (EMB — Difco) for 18–24 h. An average of 10–15 typical colonies from each water sample were selected based on morphological and physiological characteristics. Putative *E. coli* colonies were confirmed biochemically. Bacterial isolates identified as *E. coli* were stored frozen in tryptic soy broth and 15% (vol/vol) glycerol for further characterization. *E. coli* isolates were screened for antimicrobial resistance, phylotyping and enteropathogenic potential.

2.2. Antimicrobial susceptibility testing

Antimicrobial resistance was assessed using a standard disk diffusion method according to the guidelines published by the Clinical and Laboratory Standards Institute (CLSI, 2010). Bacterial suspension was adjusted to a 0.5 McFarland standard and tested against the following antimicrobials: amikacin (AMI, 30 μg), ampicillin (AMP, 10 μg), cephalotin (CFL, 30 μg), cefepime (CPM, 30 μg), cefoxitin (CFO, 30 μg), ciprofloxacin (CIP, 5 μg), gentamicin (GEN, 10 μg), nitrofurantoin (NIT, 300 μg), norfloxacin (NOR, 10 μg), sulphazotrim (SUT, 25 μg), trimethoprim (TRI, 5 μg). Reference *E. coli* strains ATCC 25922 and ATCC 35218 were used as controls. The isolates were scored as susceptible, intermediate or resistant to a given antimicrobial by the inhibition zone diameter around the disk according to CLSI (2010). Intermediate and resistant isolates were subsequently grouped in a same resistant class and categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR) (Magiorakos et al., 2012).

2.3. PCR-triplex for E. coli phylotyping

PCR assay was performed using phylogenetic group specific primers for two genes (*chuA* and *yjaA*) and an anonymous DNA fragment (TSPE4.C2) according to the method of Clermont et al. (2000). A, B1, B2 and D phylogenetic groups were determined based on the presence or absence of bands according to previously defined criteria in a triplex amplification methodology. Amplification products were inspected visually under UV light and photographed in digital image capture system (silver UVIPro, Cambridge, UK). To estimate the size of the fragments a 100 bp DNA ladder standard (Invitrogen) was used. Molecular assays included a nontemplate reaction and the clinical UPEC strain (L75A, *chua* + *yjaA* + TSPE4.C2 +) as the positive control for the phylogenetic markers (Regua-Mangia et al., 2009a). Phylotype characterization was based on the presence of the TSPE4.C2, *chuA* and *yjaA* gene markers as described.