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Research Article

Diversity of Mercury Resistant *Escherichia coli* Strains Isolated from Aquatic Systems in Rio de Janeiro, Brazil

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Escherichia coli may harbor genetic mercury resistance markers which makes this bacterial species a promising alternative for bioremediation processes. The objective of this study was to investigate phenotypic and genetic characteristics related to diversity and mercury resistance among 178 *Escherichia coli* strains isolated from residential, industrial, agricultural, and hospital wastewaters and recreational waters at Rio de Janeiro city. Genetic and conventional methods were carried out in order to determine mercury resistance. Random amplification of polymorphic DNA (RAPD-PCR) and denaturing gradient gel electrophoresis (DGGE) were used to investigate genetic variability. RAPD data revealed a high degree of polymorphism among *E. coli* mercury resistant strains and showed reproducibility and good discriminative results. DGGE typing detected diversity within the *merA* gene fragment. Our findings represent an improvement in epidemiological studies of Hg^R *E. coli* and support the evidence of nonclonal nature of mercury resistant *E. coli* strains circulating in rural and urban aquatic systems in Rio de Janeiro city.

1. Introduction

Chemical contamination of aquatic systems consists of a relevant pollution pattern causing drastic impacts on human, animal, and ecosystem health [1]. Among the various chemical contaminants, mercury plays an important role and once released in aquatic systems, mercury can resist to natural degradation processes and persist for a long time in these environments without losing its toxicity [2].

The concern about environmental contamination by this metal is due to its high toxicity, especially to the nervous system, and its bioaccumulation and biomagnification, providing persistence and wide distribution in global aquatic environment. Even regions with no mercury discharging may be affected [2–7].

Mercury toxicity to humans and other organisms is related to the chemical form to which the organisms were exposed, the route and time of exposure, dose, nutritional status, individual susceptibility, and genetic predisposition [3, 4, 6, 8]. Symptoms and contamination sources are rather different in exposure to elemental mercury, inorganic or organic mercury compounds [3, 4]. Human contamination by this metal may occur by different pathways such as vapors inhalation, contaminated food and/or water consumption, and to a lesser extent through skin contact [3, 6]. Mercury exposure triggers a series of effects including neurological, renal, cardiovascular, respiratory, gastrintestinal, hepatic, genotoxic, immunological, dermal, reproductive, and neoplasic disorders. Exposure during pregnancy may lead to

Table 1: Origin of *Escherichia coli* strains included in this study.

E. coli strains	Strains (*n*)	Aquatic system*	Sampling site	Sampling period
RM 1–RM 30 RM 33–RM 77	75	RWW	Canal do Mangue, Rio Jacaré, Canal do Cunha, Rio Faria, Rio Irajá, Canal do Meriti, Rio Sarapuí, Lagoa Rodrigo de Freitas, Lagoa da Tijuca, Lagoa de Marapendi, Rio São João	December/2009 to August/2010
RM 31-RM 32 RM 78–RM 84	09	IWW	Rio Saracuruna, Rio Imbariê, Rio Iguaçú	October/2010
RM 85–RM 110	26	AWW	Rio Vargem Grande, Córrego das Pedras	October/2010
RM 111–RM 149	29	HWW	Lagoa de Jacarepaguá	January/2011
RM 150–RM 154 RM 156–RM 179	39	RW	Parque Nacional da Praia de Ramos	January/2011

*RWW: residential wastewater; IW: industrial wastewater; AWW: agricultural wastewater; HWW: hospital wastewater, and RW: recreational waters.

malformations, mental retard, cerebral palsy, seizures, and death [3, 4, 6, 8].

Mercury resistance is one of the most studied toxic metals resistance mechanisms [7]. It has been reported that some bacteria and fungi isolated from different sources have developed resistance mechanisms that enable them to survive even in environments highly contaminated by mercury [9]. There are several described bacterial mechanisms that confer protection to harmful concentrations of mercury [10]. Among them, we highlight the mercury enzymatic detoxification, promoted by the mercuric reductase protein (MerA), which catalyze the reduction of Hg(II) to volatile Hg(0) [11, 12]. Considering the genetic of MerA expression, the Hg resistance (*mer*) operon presents a fundamental role in regulation, Hg binding, and organomercury degradation. It consists of essential genes as *merR* (responsible for the regulation of the operon), *merT/merP* (transport of mercury into the bacterial cell), *merA* (reduction of ionic mercury), and accessory genes such as *merB, merC, merD, merE, merF*, and *merG*, that encode proteins that add other skills to microorganisms [13, 14]. MerR protein can act both as a repressor and activator of transcription. In the absence of Hg^{2+}, MerR acts as repressor by binding to the *mer* operon operator region and preventing the transcription of *merTPCAD*. In presence of Hg^{2+}, it binds to one of two MerR binding sites forming a complex that acts as an activator of *mer* operon transcription [15]. Mer-mediated approaches have had broad applications in the bioremediation of mercury-contaminated environments and industrial waste streams [8, 11, 12, 16, 17].

Mercury resistance in bacteria has been observed in both Gram-positive (*S. aureus*, *Bacillus* sp.) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *Serratia marcescens,* and *Thiobacillus ferrooxidans*) [12, 16]. Mercury resistance is encoded on genetic elements such as plasmids and transposons, which contributes to horizontal dissemination among different bacteria and widespread occurrence in different bacterial groups and environments [12].

In Gram-negative bacteria, including *E. coli*, the *mer* operon has already been described [18]. However, epidemiological and genetic studies related to mercury resistance are scarce. Therefore, the investigation of the mercury resistance features has been crucial to improve bioremediation processes in contaminated environments in order to minimize human exposure and consequent adverse health effects.

In the present study, *E. coli* isolates from aquatic systems, in the city of Rio de Janeiro, Brazil, were characterized by phenotypic and genotypic traits related to mercury resistance. Bacteriological tests were carried out in order to determine mercury susceptibility, and molecular approaches based on amplification assays were used to investigate the presence and diversity of mercury resistance gene (*merA*).

2. Materials and Methods

2.1. Water Sampling. Samples were selected and grouped according to potential contamination sources in the city of Rio de Janeiro, Brazil. We studied five aquatic environments: residential, industrial, agricultural, and hospital wastewaters and recreational waters (Table 1).

2.2. Sample Collection. Collection procedure consisted of membrane filtration method with some modifications [19]. An aliquot of 60 mL of water was aspirated from the upper layer of the water column to a depth of approximately 30 centimeters with a syringe holder adapted to sterile filtration. The aspirate was filtered on a 0.22 μm cellulose acetate membrane (Milllipore) and transported under refrigeration for immediate laboratory processing.

2.3. Escherichia coli Isolation and Identification. The membrane containing the retained cells was incubated in 20 mL of tryptic soy broth (TSB, Difco) for 18–24 hr at 37°C. After a period of bacterial growth, an aliquot of the broth, diluted (1 : 10, 1 : 50, and 1 : 100) in saline 0.9% NaCl (w/v), was streaked on eosin methylene blue agar (EMB, Difco). After 18–24 h of incubation at 37°C 10–15 bacterial colonies, lactose positive and lactose negative, were selected based on morphological and physiological characteristics suggestive of *E. coli*. For confirmation of genus and species, the selected colonies were inoculated in culture medium for biochemical

identification (Probac of Brazil). *E. coli* biochemical pattern includes gas production from glucose (+), glucose utilization (+), hydrogen sulfide production (−), urea hydrolysis (−) and tryptophan deamination (−), motility (variable), indole production (+), decarboxylation of lysine (variable), and citrate (−) [20]. Bacterial cells identified as *E. coli* were stored at −20°C in TSB plus 15% glycerol (v/v) until analysis. This study included a total of 178 *E. coli* isolates (RM 1 to RM 179) (Table 1).

2.4. Mercury Resistance Phenotype. In order to classify *E. coli* as resistant or sensitive to mercury, each strain was tested on nutrient agar (NA, Difco) supplemented with 5 μM of Hg^{2+}. Evidence of bacterial growth after a period of 24–48 h at 37°C allowed to classify *E. coli* strains as Hg resistant (Hg^R). *E. coli* ATCC 35218 (Hg resistant) and *E. coli* ATCC 23724 (Hg susceptible) were used as control strains. When no growth was observed, a strain was considered as sensitive. These tests were done in duplicate.

2.5. Minimal Inhibitory Concentration (MIC). MIC determination was performed following the methodology described by Andrews [21] with some modifications. Overnight cultures of the isolates in nutrient broth (NB, Difco) containing 1 μM Hg were adjusted in saline NaCl 0.9% (w/v) in order to contain 1.5×10^9 bacterial cells/mL (McFarland 0.5). An aliquot of 50 μL was inoculated in nutrient agar plates containing 10 to 40 μM Hg. After 24–48 h at 37°C, the MIC value was determined by observing bacterial growth on agar plates in the presence of the lowest Hg concentration. MIC tests were performed with those *E. coli* strains exhibiting mercury resistance phenotype ≥5 μM Hg. All experiments were performed in duplicate.

2.6. merA Detection. All strains were screened for the presence of *merA* sequence by PCR amplifications as described by Ní Chadhain and colleagues [22], with some modifications. Each reaction was carried out in 25 μL PCR mixture containing 3 μL of bacterial DNA obtained through thermal extraction of 18–24 h bacterial growth in tryptic soy broth (TSB, Difco), 2.5 μL of 10X buffer (Invitrogen), 2 mM $MgCl_2$ (Invitrogen), 0.2 mM dNTP (Invitrogen), 30 μM of each primer, and 1 U of Platinum *Taq* DNA polymerase (Invitrogen). The pair of primers used was A1s-n.F (5′-TCCGCAAGTNGCVACBGTNGG-3′) and A5-n.R (5′-ACCATCGTCAGRTARGGRAAVA-3′). PCR reaction was conducted in *Mastercycler Personal* thermocycler (Eppendorf) under the following amplification conditions: initial denaturing step at 94°C for 5 min, followed by 45 cycles at 94°C for 10 sec, 68°C for 40 sec, and 72°C for 1 min with a final extension at 72°C for 7 min. Approximately 10 μL of the resulting amplification products was added to 2 μL of running buffer (*gel loading buffer*, Invitrogen) and separated by electrophoresis on agarose gel at 1.3% concentration (w/v) prepared in Tris-Borate-EDTA 0.5X (5X-0.89 M Tris-HCl (LGC Biotech) 0.89 M boric acid (Merck) and 0.024 M EDTA (LGC Biotech) (pH 8.4)) at a constant voltage of 70 V. Electrophoresis gel was stained with 0.5 μg/mL ethidium bromide solution (Invitrogen) over a period of 15 min and washed in distilled water for about 30 min. Gel was visually inspected by using an ultraviolet light transilluminator (UVITec, Cambridge, UK) 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. *E. coli* strains ATCC 35218 (Hg resistant) and ATCC 23724 (Hg sensitive) were used as controls.

2.7. Random Amplification of Polymorphic DNA (RAPD-PCR). RAPD-PCR analysis was performed according to the methodology described by Pacheco and colleagues [23]. Each reaction was carried out in a 30 μL PCR mixture containing 2 μL of bacterial DNA, 3 μL of 10X buffer (Invitrogen), 250 μM each dNTP (Invitrogen), 3 mM $MgCl_2$ (Invitrogen), 1 U of *Taq* DNA polymerase (Invitrogen), and 30 μM of each primer. Primers used were 1247 (5′-AAGAGCCCGT-3′), 1254 (5′-CCGCAGCCAA-3′), 1290 (5′-GTGGATGCGA-3′), and A04 (5′-AATCGGGCTG-3′). The reaction was conducted in a *Mastercycler Personal* thermocycler (Eppendorf) under the following amplification conditions: an initial denaturing step at 94°C for 1 min, followed by 4 cycles at 94°C for 4 min, 37°C for 4 min, and 72°C for 4 min, 30 cycles at 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min with a final extension at 72°C for 10 min. Reaction products were analyzed by electrophoresis in 1.5% agarose gels and stained with ethidium bromide. RAPD profiles were inspected visually and defined according to the presence or absence and intensity of polymorphic bands. A 1 kb DNA ladder was used as a molecular weight marker (GIBCO, BRL, Gaithersburg, MD, USA). Semiautomated analysis used the UVI Soft Image Acquisition and Analysis Software, program UVIPro bandmap version 11.9 (UVItec, Cambridge, UK). Cluster analysis was done by using the unweighted pair group method with arithmetic averages (UPGMA) of the Image Analysis System. The percentages of similarity were estimated by the Dice coefficient. The reproducibility of the RAPD amplifications was assessed using the selected primers with different DNA samples isolated independently from the same strain and amplified at different times.

2.8. Denaturing Gradient Gel Electrophoresis (DGGE). DGGE analysis was performed according to the methodology described by Muyzer and colleagues with some modifications [24]. AxyPrep DNA Gel Extraction kit(Axygen Biosciences) was used for purificating the PCR-*merA* DNA fragment (285 bp). For PCR-DGGE reaction a final volume of 25 μL in amplification reactions containing 3 μL of purified DNA, 2.5 μL 10X buffer (Invitrogen), 2 mM $MgCl_2$ (Invitrogen), 0.2 mM dNTPs (Invitrogen), 30 μM of each primer, 1% formamide, and 1 U of Platinum *Taq* DNA polymerase (Invitrogen) was used. The pair of primers for amplification was A1s-n.F (5′-TCCGCAAGTNGCVACBGTNGG-3′) and A5-n.R (5′-ACCATCGTCAGRTARGGRAAVA-3′). The reaction was conducted in *Mastercycler Personal* thermocycler (Eppendorf) and programmed for an initial denaturation of 94°C for 5 min followed by 45 cycles of 94°C for 10 sec, 68°C for 40 sec, and 72°C for 1 min, with a final extension of 72°C for 7 min. Approximately 25 μL of amplified PCR product was added to 15 μL of DNA electrophoresis dye (0.005 g

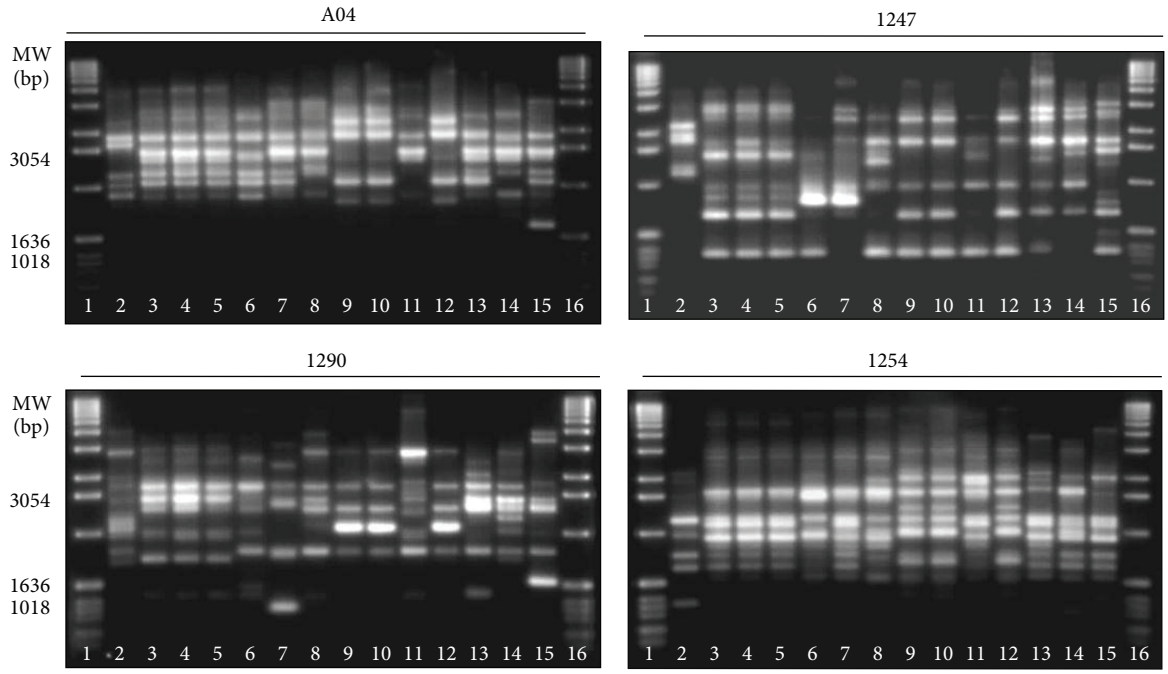

Figure 1: RAPD-PCR profiles of representative *E. coli merA*+ strains obtained by using 4 different primers (A04, 1247, 1290, and 1254). Lanes 1, 16: 1 Kb DNA ladder; Lane 2: strain RM 1; Lane 3: strain RM 7; Lane 4: strain RM 8; Lane 5: strain RM 9; Lane 6: strain RM 17; Lane 7: strain RM 20; Lane 8: strain RM 31; Lane 9: strain RM 37; Lane 10: strain RM 44; Lane 11: strain RM 45; Lane 12: strain RM 46; Lane 13: strain RM 61; Lane 14: strain RM 150; Lane 15: strain RM 165.

Bromophenol blue, 0.005 g xylene cyanol, 7 mL glycerol P.A., and 3 mL deionized water) and ran on a polyacrylamide gel (8% w/v of acrylamide/bisacrylamide ratio 37.5 : 1) with a linear denaturant gradient ranging from 55% to 80% (where 100% is a solution of 7 M urea and 40% formamide v/v). Electrophoresis was performed in equipment using the *Dcode Universal Mutation System* (BIO-Rad) and conducted at constant voltage of 100 V at 60°C for 6 h in 0.5X Tris-acetate (10 mM Tris-acetate, 5 mM Sodium Acetate, 25 mM EDTA, and pH 7.4). After electrophoresis the gel was stained with Sybr Green (Molecular Probes, OR, USA) for 30 minutes and visualized under UV transilluminator. The reproducibility of the assay was tested by loading three PCR products for each sample on DGGE gels.

3. Results

3.1. Mercury Resistance Phenotype and Minimal Inhibitory Concentration (MIC). A total of 164 strains were classified as mercury resistant (Hg^R) and represented 92.1% of the *E. coli* isolates (164/178). All Hg^R exhibited the Hg MIC value of 10 μM.

3.2. PCR Amplification of merA Gene. Among *E. coli* strains analyzed in this study, 14 harbored the 285 bp *merA* gene fragment described by Ní Chadhain and colleagues [22]. *E. coli* strains carrying the 285 bp *merA* gene corresponded to 14.7% (11/75) of the isolates obtained from residential wastewaters samples, 11.1% (1/9) from industrial wastewaters samples, and 6.9% (2/29) from hospital wastewaters samples.

3.3. Random Amplification of Polymorphic DNA (RAPD-PCR). The diversity within the *E. coli merA*+ strains was investigated by RAPD-PCR using the primers A04, 1247, 1290, and 1254 (Figure 1). RAPD typing revealed a high degree of diversity among *E. coli* strains. Reactions performed with primers A04, 1247, 1290 (60% GC, each), and 1254 (70% GC) resulted in 11, 10, 10, and 10 different RAPD profiles, respectively. The total number of polymorphic bands was 5–9 bands (A04), 4–11 bands (1247), 5–10 bands (1290), and 8–12 bands (1254) ranging from 600–4100 bp, 200–5600 bp, 450–8000 bp, and 250–9000 bp, respectively. There was no direct correlation between higher G+C content and the ability of the primer to detect polymorphism. The different primers used to investigate the overall chromosomal relatedness among *E. coli* strains were strongly correlated. The cluster analysis revealed a bacterial population arranged into separate branches or small clonal groups, exhibiting Dice similarity index ranging from 6–100%, 18–100%, 6–100%, and 6–100% for primers 1290, 1254, 1247, and A04 (Figure 2), respectively. Close relatedness was specially observed among *merA*+ *E. coli* strains isolated from the same aquatic system (Table 2, Figure 2). Identical RAPD profiles were observed among residential wastewaters isolates: RM 7, RM 8, and RM 9, isolated from Canal do Cunha, and RM 37, RM 44, and RM 46 from Lagoa Rodrigo de Freitas.

3.4. Denaturing Gradient Gel Electrophoresis (DGGE). Electrophoresis technique on denaturing gradient gel enabled the detection of variability within the 285 bp gene fragment associated with mercury resistance (*merA*). Supporting the results obtained from RAPD-PCR, RM 7, RM 8, and RM

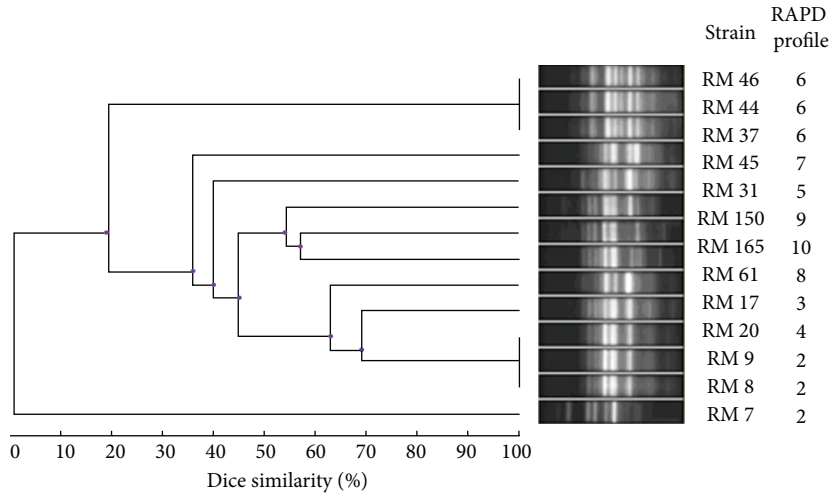

Figure 2: Dendrogram generated by the Dice coefficient and clustering by unweighted pair group method with arithmetic mean and respective RAPD profiles of *merA*+ *Escherichia coli* isolates using 1254 primer.

Table 2: Genetic and phenotypic traits of *E. coli* strains carrying the 285 bp *merA* fragment according to aquatic systems and sampling sites.

E. coli strain	Aquatic system*	Sampling site	MIC	RAPD profile			
				A04	1247	1290	1254
RM 1	RWW	Canal do Mangue	10 μM	1	1	1	1
RM 7		Canal do Cunha	10 μM	2	2	2	2
RM 8		Canal do Cunha	10 μM	2	2	2	2
RM 9		Canal do Cunha	10 μM	2	2	2	2
RM 17		Rio Irajá	10 μM	3	3	3	3
RM 20		Rio Irajá	10 μM	4	4	4	4
RM 31	IWW	Rio Iguaçú	10 μM	5	5	5	5
RM 37	RWW	Lagoa Rodrigo de Freitas	10 μM	6	6	6	6
RM 44		Lagoa Rodrigo de Freitas	10 μM	6	6	6	6
RM 45		Lagoa Rodrigo de Freitas	10 μM	7	7	7	7
RM 46		Lagoa Rodrigo de Freitas	10 μM	8	6	6	6
RM 61		Lagoa de Marapendi	10 μM	9	8	8	8
RM 150	HWW	Lagoa de Jacarepaguá	10 μM	10	9	9	9
RM 165		Lagoa de Jacarepaguá	10 μM	11	10	10	10

*RWW: residential wastewater; IW: industrial wastewater; HWW: hospital wastewater.

9 isolates also showed identical DGGE pattern (Figure 3). Despite the diversity observed, no significant differences among the DGGE band patterns were observed.

4. Discussion

4.1. Mercury Resistance Phenotype and Minimal Inhibitory Concentration (MIC). Many studies have been conducted in order to determine the mercury resistance in environmental bacteria by testing the minimum inhibitory concentration [25–28]. There is not a standard protocol for determining the MIC of heavy metals. Liquid and/or solid media with different chemical compositions have been commonly used for these assays, as well as variation of metals concentrations. Methodology itself may offer some obstacles such as precipitation and volatilization of the solution and complexes between the metal and culture medium components. These variations, if not minimized before its application, may directly influence the result obtained [26]. So, it is very difficult to compare the obtained results with previous studies because of the great diversity of MIC values and the procedures adopted, especially considering the broad spectrum of mercury resistant bacteria that require specific conditions for growing and laboratory processing.

The ubiquity of bacterial mercury resistance has been observed in environments worldwide and is supposed to be the result of external interference by humans and other animals through environmental contamination for several years [5, 12, 26]. There were no reports about mercury contamination in the sampling sites; however, Hg resistance was widely detected. Bacterial resistance to mercury present in the environment is considered as one of many examples of

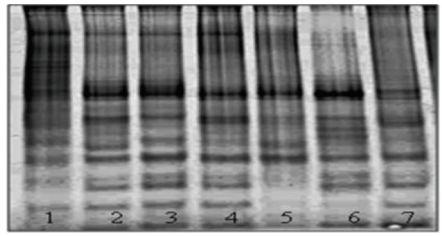

Figure 3: DGGE profiles of PCR-amplified *merA*+ gene fragment from *E. coli* strains. Lane 1: strain RM 1; Lane 2: strain RM 7; Lane 3: strain RM 8; Lane 4: strain RM 9; Lane 5: strain RM 17; Lane 6: strain RM 20; Lane 7: strain RM 31.

genetic and physiological adaptation of microbial communities exposed to contaminants. Several factors have been found to contribute to this phenotype in the rural and urban areas including the use of mercury-based fungicide in the paper industry, agriculture, and hospital disinfectants. These factors may encourage selective activities and result in mercury resistance in open environment [29]. Additionally, toxic metal resistance genes are commonly found in environmental bacteria, and these genes may confer coresistance or crossresistance to antimicrobial drugs codified on the same genetic element [26, 30]. So, selection of microbial communities exposed to toxic levels of the metal or submitted to the coselection mechanisms has led to high rates of circulation of these resistant bacteria in aquatic systems [31, 32].

4.2. PCR Amplification of merA Gene. The genetic system evolved as *mer* operon is the only well-known bacterial mercury resistance system with high yield transformation of its toxic target into volatile nontoxic forms [27, 31, 33–35], particularly in Gram-negative bacteria [14, 22]. The *mer* locus is found to be widely distributed among bacterial lineages, and *mer*-like sequences have been described. Several biochemical mechanisms are identified, and the complexity among the ecological niche of mercury-resistant microbes is still not fully described [10, 35]. *merA* plays a key role on mercury resistance of bacterial community exposed to mercury contamination, but the combinatorial action of genetic determinants seems to confer a broad spectrum mercury detoxification system [10, 35]. So, the involvement of additional genetic determinants not investigated here, acting as effectors or regulators genes, must be considered for the expression of mercury resistance phenotype among *merA* negative *E. coli* strains. *merA* gene was detected *in E. coli* isolated from residential wastewaters (11/75), industrial wastewaters (1/9), and hospital wastewaters (2/29). The higher frequency of *merA*+ *E. coli* strains obtained from residential wastewaters compared to industrial and hospital wastewaters may be related to several factors such as the representative sampling of each area investigated and involvement of additional genetic determinants as well as related to the intrinsic characteristics of the rural and urban locations.

4.3. Random Amplification of Polymorphic DNA (RAPD-PCR). RAPD-PCR is a recognized powerful tool showing high discriminatory potential, reproducibility, sensibility, and specificity under well-standardized protocols. Random amplification of polymorphic DNA (RAPD) has been successfully used as a molecular typing system for studies on diversity of *E. coli* population [23, 36].

RAPD typing revealed levels of polymorphism that are consistent with previously reported observations for *E. coli* and has been attributed to the high plasticity of this bacterial species. Several molecular approaches mainly based on genetic techniques have been successfully applied in order to assess the clonal nature and variability within species [23, 36]. The occurrence of distinct patterns of *E. coli* phylogenetic distribution provides evidence of both vertical and horizontal transmission [37–39]. The mechanisms of genetic diversification contribute to *E. coli* evolution and creation of new variants, as this bacterial species is often subjected to DNA rearrangements, excisions, transfers, and acquisitions [37, 40]. There are several highly adapted clones that have acquired specific virulence elements which confer an increased ability to adapt to new niches. Such plasticity may confer ability to acclimate environmental bacteria to new niches allowing these microorganisms to become members of microbial communities in a variety of environments, even facing conditions very different from their primary habitat [36, 38, 39, 41]. RAPD-PCR approach was used to investigate the overall chromosomal relatedness among *merA*+ strains and revealed a high genetic diversity population suggesting that mercury resistance is widely dispersed in *E. coli*. The observed genotypic diversity led us to suppose that, in Rio de Janeiro, *merA*+ *E. coli* isolates consist of nonrelated epidemiological strains and may represent distinct evolutionary lineages. Despite the genetic variability, clustering analysis revealed that the degree of diversity was to a lesser extent among *E. coli* strains obtained from the same aquatic environment evidencing the circulation of closely related strains.

4.4. Denaturing Gradient Gel Electrophoresis (DGGE). DGGE fingerprinting is a technique widely used in microbial ecology studies and has been focused on studies of genetic diversity and bacterial communities from several environments [17, 42]. Variability within *merA* gene has been described, and diverse MerA protein homologs have been identified in both archaeal as well as bacterial genomes but not in eukaryal genomes [17]. The increased complexity of *mer* operons can be attributed to the gradual addition of functions involved in the regulation of the operon by Hg, Hg transport, and organomercury resistance [17]. The diversity *of merA* gene in Gram-negative and Gram-positive bacteria has been accessed by several approaches including those using restriction fragment assays [27, 31, 33, 34]. In all these studies, a high genetic variability was detected in *merA* determinant carried by bacterial species from different environments. However, RFLP technique is limited since it relies on specific target, requiring prior knowledge of the sequences to be analyzed. In the present study, DGGE was used to investigate the *merA*+ variability among *E. coli* mercury resistant.

DGGE typing revealed diversity within the 285 bp *merA* fragment corroborating previous findings that described

the occurrence of genetic exchanges in mercury resistance gene as a result of addition, rearrangements, excisions, and horizontal transfer.

Ní Chadhain and colleagues [22] developed a protocol using degenerated primers and detected high diversity within *merA* sequence from evolutionary distinct Gram-bacteria. In our study, this methodology allowed the detection of variability in the 285 bp *merA* fragment among 14 *E. coli* strains (Figure 3). These results are in agreement with previous findings regarding the widespread occurrence and diversity of mercury resistance markers among distinct microbial populations from several environments, including soils and sediments, aquatic systems, animals, and clinical isolates [13, 14, 27, 28, 31, 32]. The high plasticity found in the bacterial genome contributes to the diversity and dissemination of genetic markers favoring its circulation in geographically dispersed environments, even between distinct evolutionary lineages.

E. coli isolates sharing similar RAPD profiles were found to exhibit the same *merA* DGGE pattern suggesting the circulation of conserved or partially conserved *merA* sequence among closely related strains. The molecular approaches used as fingerprint tools were found to be accurate and useful methods in distinguishing between closely related bacteria. The obtained results are relevant to our understanding on the characteristics of mercury resistant *E. coli* circulating in natural environments in aquatic systems in Rio de Janeiro. Our findings substantially expand our knowledge about *mer* evolution and biodiversity of these microorganisms, and contribute to studies on bioremediation process and environmental management of Hg contamination.

5. Conclusions

The present study detected a wide dissemination of *E. coli* isolates resistant to mercury in distinct aquatic systems in the city of Rio de Janeiro possibly due to selective activities with varying patterns of exposure to Hg. Genetic analysis of *merA*+ strains revealed high degree of diversity among the bacterial population indicating that mercury resistance is widely dispersed in *E. coli*. These findings suggest that, in the city of Rio de Janeiro, *merA*+ *E. coli* may constitute bacterial communities epidemiologically independent and may represent distinct evolutionary lineages. The variability detected within the 285 bp *merA* fragment possibly reflects the occurrence of specific genetic events. *E. coli* strains sharing RAPD profile and DGGE band pattern reinforce the hypotheses of circulation of conserved *merA* sequence among closely related strains. In the light of the pathogenicity attributed to *E. coli* population, more accurate analyses are required for applications in bioremediation processes.

Conflict of Interests

The authors have declared that no conflict of interests exists.

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