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Community, environment and hospital associated beta-lactamase resistance in Escherichia coli in Salvador, Brazil

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Article

Keywords: extended spectrum, carbapenemase, resistance genes, water-borne disease, fecal contamination, one health

Posted Date: July 21st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6334420/v1

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Additional Declarations: No competing interests reported.

Abstract

The tendency of enteric bacteria to acquire resistance to antibiotics of last resort has placed them at the top of the WHO list of priority pathogens. Their presence as commensal in the human gut also means enterics such as *Escherichia coli* easily contaminate multiple environments where sanitation is poor. We compared the presence of *E. coli* resistant to 3rd generation cephalosporins and carbapenem antibiotics across the compartments of fecal carriage, local surface waters and a hospital in the same urban community in Brazil. Species identification and antimicrobial sensitivity profiles were confirmed using MALDI-TOF and Vitek 2. PCR amplification was used to assay 17 β-lactamase genes. Resistance profiles of fecal samples from healthy residents and river water showed most similarity, but samples collected from the river had the highest diversity of β-lactamase genes and presented significant divergence from the other compartments. Hospital isolates had much higher rates of carbapenem resistance. Despite grossly elevated indicators of human fecal contamination, there is not a simple relationship between human fecal carriage, the aquatic resistome and hospital infections. Surface waters in an urban Brazilian site may serve as an environmental reservoir of antimicrobial resistance.

Introduction

Antimicrobial resistance (AMR) is increasingly a major challenge for public health, due to the emergence of resistance to commonly used antibiotics and especially to last line agents[1]. Increasing rates of AMR render many antibiotics ineffective and result in increased morbidity and mortality. Among the six main pathogens associated with resistance and lethality, *Escherichia coli* is described as the most important [2]. The growing global issue of AMR has also contributed significantly to public health economic spending. It is estimated that if an effective action plan is not implemented, the annual mortality rate from bacterial infections by some estimates is expected to reach 10 million deaths per year by 2050, more than deaths caused by cancer[3]. The problem is acute in Brazil [4].

Antimicrobial-resistant bacterial isolates in the hospital are associated with infections with a high rate of lethality due to the capacity of these isolates to acquire resistance through the production of extended-spectrum β-lactamases (ESBL) [5]. In the community, resistance is related to colonization of individuals due to the use of antimicrobials and potentially to acquisition from food, water or other individuals and pets [6]. Resistance is accelerated by the inappropriate use of antibiotics. In the environment, the presence of resistant bacteria is mainly due to lack of adequate water and sewage treatment [7]. Poor sanitation results in fecal contamination of aquatic environments, making them reservoirs of antimicrobial resistance genes (ARGs) [8,9]. While bacterial transformation may occur with naked DNA, the most efficient transformation is through directed transfer by R plasmids [10,11]. This is a process that requires cell-cell contact which is promoted under conditions of poor sanitation. *E. coli* is considered the main enteric bacteria that disseminates resistance genes through horizontal transfer in these environments [12].

We aimed to describe the patterns of antimicrobial resistance in *E. coli* isolates from a community, river and hospital in the same geographic region of the city of Salvador, Bahia, Brazil. We also compared the AMR profile in *E. coli* found in each of these interacting compartments. Although direct transfer from one compartment to another cannot be shown with these data, the prevalence of these bacteria and their genes may reflect a relationship or lack thereof among them.

Results

Fecal contamination markers

By Coliscan tracking of coliforms and MST analysis, collection points P1 and P2 had the lowest *E. coli* and general Bacteroides spp. contamination (Fig. 3). The points of greatest contamination for all fecal biomarkers were the points that follow the river flow to its mouth and the highest population density (P5, P6 and P7). The concentrations of total coliforms and *E. coli* ranged from 1 to greater than 1,000 CFU/mL. Likewise, the markers for human fecal contamination, *Bacteroides* spp. and *Lachnospira* spp., also showed a higher number of copies at these three points, indicating the presence of human fecal contamination more significantly at these points which was anticipated.

Escherichia coli isolates resistance profiles

Fecal samples: From 1,134 fecal samples, 329 were selected at random. Presumptive cefotaxime-resistant *E. coli* isolates were identified from 113, and 42 of these were 3rd generation cephalosporin- or carbapenem- resistant by Vitek-2. Most of these isolates (39/42) were also resistant to 4th generation cephalosporins and 83.33% (35/42) resistant to ciprofloxacin. The isolates were most susceptible to gentamicin and piperacillin/tazobactam with 4.76% (2/42) resistant. Finally, no isolate showed resistance to the tested carbapenems (Fig. 4).

River samples: From 28 collections, a total of 59 *E. coli* isolates resistant to ceftriaxone were collected. Based on VITEK 2 susceptibility tests, the selected *E. coli* river isolates from had high prevalence of resistance to the 3rd and 4th generation cephalosporins ceftriaxone 100% (59/59), ceftazidime 100% (59/59) and cefepime 74.57% (44/59). All were also resistant to ciprofloxacin 100% (59/59). The rates of resistance to amoxicillin/clavulanic acid, gentamicin and piperacillin/tazobactam were 74.57% (44/59), 22.03% (13/59), 13.55% (8/59), respectively. The isolates showed low levels of resistance to meropenem and ertapenem at 13.55% (8/59) and 3.38% (2/59), respectively (Fig. 4).

Hospital samples: In comparison with *E. coli* isolates from the water and feces, hospital isolates showed the highest resistance to the antimicrobials tested, especially to the carbapenems. All hospital *E. coli* isolates (23/23) were resistant to amoxicillin/clavulanic acid, ciprofloxacin and cephalosporins (ceftriaxone, ceftazidime and cefepime). Resistance to meropenem and ertapenem was observed in 65.2% (15/23) and 60.9% (14/23) of the isolates, respectively. The rates of gentamicin and piperacillin/tazobactam resistance were 82.6% (19/23) and 69.6% (16/23), respectively (Fig. 4).

Antibiotic resistance gene profiles

We analyzed the resistance profile of the 124 identified *E. coli* isolates resistant to 3rd generation cephalosporins for the presence of 17 ARGs. β-lactamase genes were detected in 41% of these isolates. The largest number of single genes and gene combinations were present in samples from the river. (Fig. 5a and 5b). Among the 124 isolates, 21% (26/124) carried a single β-lactam resistance gene. The most frequent genes identified were $_{bla}$OXA1/4/30 and $_{bla}$CTX-M-1, both at 31% (8/26), followed by $_{bla}$VIM-1/2 at 12% (3/26). Only *E. coli* isolates from water samples were positive for $_{bla}$OXA-1/4/30, $_{bla}$VIM-1/2 and $_{bla}$KPC-1/2/3/4 genes and a single strain of *E. coli* from the feces of a resident of the Pirajá community was positive for $_{bla}$CTX-M-9 (Fig. 5a).

Among the 124 *E. coli* isolates, 20% (25/124) were positive for multiple genes. The most frequent were Ambler class A β-lactamases at 20% (5/25) $_{bla}$SHV-1 + $_{bla}$TEM-1/2, followed by $_{bla}$SHV-1 + $_{bla}$TEM-1/2 + $_{bla}$OXA-1/4/30 and $_{bla}$CTX-M1 + $_{bla}$CTX-M-9 both at 16% (4/25). Only fecal samples had the combination SHV-1+TEM-1+OXA-1/4/30. One river isolate harbored 6 different genes (Fig. 5b). No isolates were positive for $_{bla}$OXA-48, $_{bla}$OXA-23, $_{bla}$BKC-1 and $_{bla}$GES.

Discussion

In a community with poor sanitation, a nearby hospital, and ample surface water these compartments are unlikely to be microbially isolated from one another. An assessment of water quality from the Cobre River showed heavy contamination with human feces, and there was multidrug resistant *E. coli* in all compartments. A clear path of transmission between human feces, surface waters and the hospital, however, is impossible to trace with the data we have here. Exactly what is exchanged and how cannot be directly identified, but an analysis of the composition of resistance profile of each can begin to suggest possible relationships or a lack thereof.

While the antibiotic resistance profile of the local human fecal carriage is most similar to that of the river (Fig. 4), the profile of resistance genes was distinct for each compartment (Fig. 5). Isolates with the OXA-1/4/30 and VIM-1/2 β-lactamases singly or in combination, for example, were found only in river samples except for five human fecal samples. CTX-M-9 was observed exclusively in hospital isolates, except for one community isolate. The combination SHV-1+TEM1+ OXA-1/4/30 was only found in human fecal samples. The highest prevalence of resistance to broad spectrum β-lactams was present in *E. coli* from hospital infections. By contrast, the widest variety of genes tested was from the Cobre River samples. This suggests that there is some isolation either of transmission, maintenance, or development of certain resistant strains between each of the compartments. By the same token the most highly resistant hospital strains do not easily make it back to the community or the river (Fig. 5).

The selected antibiotic resistance genes were detected in 41% of *E. coli* isolates, and blaCTX-M-1, blaSHV-1, blaTEM1 and blaOXA-1/4/30 were the most prevalent in this study. This is similar to a previous study in Salvador in bacteremic patients where Enterobacterales isolates also had a higher

frequency for these genes [13]. β-lactam resistance in the other 60% of isolates may either be due to β-lactamase genes not tested or bacteria with the AmpC β-lactamase. blaCTX-M-1 was the most prevalent (31%) which is consistent with CTX-M-types being the most widespread enzymes [14]. In Enterobacterales, this dissemination is due to carriage by conjugative plasmids [11], whose transfer is enhanced by cell contact facilitated by poor sanitation.

Among the isolates that contained multiple genes, the most frequent combinations were blaSHV-1 + blaTEM-1/2 (20%), followed by blaSHV-1 + blaTEM-1/2 + blaOXA-1/4/30 (16%) and blaCTX-M1 + blaCTX-M-9 (16%). Since the 1990s, the majority of ESBLs identified have been derived from blaTEM-1/2 or blaSHV-1 types. Also, strains producing these enzymes are distributed mainly in China, Central and South America [15]. The occurrence of the blaNDM gene in each compartment, though infrequent, is of particular concern since bacteria with this gene are carbapenem-resistant, which increases the impact of their dissemination [16].

Multidrug resistant *E. coli* strains are commonly observed in water samples from low income countries [6,17,18]. Since over 80% of antibiotics are dispensed to outpatients [19], it follows that sanitation has an important role in the dissemination of antibiotic resistant Enterobacterales. The contribution of fecal contamination to aquatic environments not only contributes to the local microbiota but also transforms these environments into reservoirs, nurseries, and potential disseminators of resistance genes [20], that in some cases are then amplified in the hospital setting. Rural and urban surface waters in Brazil are known for their potential sources of multidrug-resistant bacteria. In the Cobre River, this contamination phenomenon was no different from what we found in other previously evaluated urban water collections in Salvador [8,21]. Improved sanitation needs to be included in the list of antimicrobial stewardship objectives.

Limitations

Comparison between compartments is not on an equal basis. Samples from the Cobre River and Subúrbio Hospital were collected longitudinally over a year. Fecal samples were collected only once from each individual over the span of approximately one month: The samples collected over time may have provided more opportunity to detect a wider range of resistance genes. Samples collected from hospital infections did not represent colonization like the samples from healthy people in the community. Hospitalized patients may have received broad spectrum antibiotics. In addition, none of the samples from infections were from feces, and most infections were due to single organisms, so the microbiota at different sampling sites may have influenced available resistance gene profiles. We still feel that the influence of these factors would not significantly affect the substance of the conclusions presented.

Conclusions

Within a single region, multidrug-resistant *E. coli* were found in Cobre River, feces of residents of the Pirajá neighborhood and from hospitalized patients. In the Cobre River, all points demonstrated some

type of contamination, including human fecal contamination and may serve as an environmental reservoir of AMR. Antimicrobial resistance genes blaCTX-M, blaSHV, blaTEM and blaOXA were the most prevalent ESBLs in this study. With some caveats, the greatest variety of β-lactamase genes were found in the river suggesting this as a potential source for distribution.

Materials and Methods

Study Sites: The present study was carried out in the Pirajá neighborhood of Salvador, with the Cobre River bordering the neighborhood, and a large general hospital (313 beds) in the same region along the river (Fig. 1).

Microbial source tracking (MST): The 500 mL samples collected from each site on the Cobre river were filtered through a 0.22 µm pore mixed cellulose filter (EMD Millipore Corporation, Billerica, MA) using a Nalgene filter funnel unit (Thermo Scientific, Waltham, MA). The filters were thoroughly dried and then stored at -20 °C until used. DNA was extracted by a phenol/chloroform procedure. The extracted DNA was used to identify and quantify bacteria indicative of human fecal contamination with targets for the general Lachnospira spp. and Bacteroides spp and human sources. The qPCR assays were performed in final reactions of 25 µL and in duplicate[8]. Each assay was performed in duplicate and the results averaged.

Sampling and screening for resistant *E. coli*: Feces. The study took advantage of a community-based program of 1,134 stool examinations for parasitic diseases [22]. Stool samples were selected based on a random number protocol for AMR assays. From each fecal sample, 1g was collected using a sterile swab placed in Cary-Blair transport medium and plated on MacConkey Agar (Merck, Darmstadt, Germany) with 2 µg/mL of cefotaxime(12)[23]. Bacterial colonies morphologically characteristic of *E. coli* were selected for inoculation on Triple Sugar Iron agar (TSI) (Neogen, Lansing, Michigan, USA). All glucose fermenting bacteria in the TSI were re-isolated on Tryptone Soy Agar (TSA) (Neogen, Lansing, Michigan, USA) and sent for definitive identification by MALDI-TOF and Vitek 2 (Fig. 2).

River water. Samples were collected while facing upstream at approximately 10 m from shore at seven points along the course of the Cobre River at three-month intervals from October 2021 to August 2022. All collections were made between 8 am and 12 pm with sterile 500 mL glass bottles. The samples were placed in a cooler with ice packs until processing. One mL samples were removed from water at each point, mixed with Coliscan Easygel media (Micrology Laboratories, Goshen, IN), and plated. After 24-48 hours of incubation at 25-30 °C, *E. coli* and coliform colonies were counted manually in duplicate up to 1,000 CFU/mL. *E. coli* was differentiated from other coliforms by their blue/purple or pink color, respectively.

Collected water was diluted (1:1, 1:10, 1:100 and 1:1000), and 100 µL plated on MacConkey Agar (Merck, Darmstadt, Germany) with 2 µg/mL of cefotaxime (SIGMA®, United States) [23]. The plates were incubated for 24 hours at 36 ºC ± 2 ºC. Colonies with morphological characteristics suggestive of enterobacteria

were selected for TSI (Triple Sugar Iron) agar medium (Neogen, Lansing, Michigan, USA) to further screen for *E. coli*. All glucose fermenting bacteria in the TSI were re-isolated on TSA Agar (Tryptone Soy Agar) (Neogen, Lansing, Michigan, USA) and sent for definitive identification by MALDI-TOF and Vitek.

Hospital. From the hospital microbiology laboratory, all (n=23) *E. coli* isolates resistant to 3rd generation cephalosporins and confirmed by Vitek were collected weekly (March to December 2022). All were from deep infections of sites that should otherwise be sterile in hospitalized patients, and none were from stool. All ceftriaxone-resistant *E. coli*, confirmed by Vitek were collected over the 11 months of the study from the hospital's microbiology laboratory.

Ethical Approvals. The study protocol was approved by the Brazilian National Commission on Ethical Research CAAE: 42424915.9.0000.0040 and Tulane University IRB# 2019-1799. All research was performed in accordance with relevant guidelines and regulations. The sampling was random and anonymized using discarded waste materials, informed consent was not required. Where personal health information was required under an unrelated part of the project, informed consent was required.

Microbial identification and AMR confirmation: The species identification was confirmed by MALDI-TOF® (Matrix Assisted Laser Desorption Ionization) (VITEK®, Biomérieux, France) and antimicrobial susceptibility profile determined by VITEK®-2 automated system (Biomérieux, France), following the standardization criteria of the Brazilian Committee on Antimicrobial Susceptibility Testing (BR-CAST) for enterobacteria.

β-lactamases gene identification: Polymerase chain reaction (PCR) (multiplex and singleplex protocols) was used to amplify β-lactamase genes which were subsequently analyzed by agarose gel electrophoresis: Multiplex I ($_{bla}$TEM-1, $_{bla}$TEM-2, $_{bla}$SHV-family, $_{bla}$OXA-1, $_{bla}$OXA-4 and $_{bla}$OXA-30)(13); multiplex II ($_{bla}$CTX-M-1, $_{bla}$CTX-M-2 and $_{bla}$CTX-M-9 group)(13); multiplex III ($_{bla}$VIM-1, $_{bla}$VIM-2, $_{bla}$IMP-family, $_{bla}$KPC-1 to 4)(13)[24]; multiplex IV ($_{bla}$GES-1 to 9, $_{bla}$GES-11 and $_{bla}$OXA-48-like) [24] and singleplex for $_{bla}$NDM [25].

Declarations

Acknowledgments

This work was supported by the Brazilian Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) and the Brazilian National Council for Scientific and Technological Development (CNPq) and the National Institutes of Health USA [R01AI121330]. The Gonçalo Moniz Institute, Oswaldo Cruz Foundation Bahia provided some transportation and facilities. We thank the Bahiana School of Medicine and Public Health for use of the clinical laboratory for the microbiological analyses.

Author contributions

Vanessa T. Moretto, Conceptualization, Investigation, Methodology, Formal analysis, Writing – review & editing; Ana Beatriz Purificação, Investigation; João Ricardo Filho, Investigation; Lee Senhorinha Almeida, Investigation; Camila Freitas Chaves; Investigation; Adriano Coelho, Visualization; Rafael Hermida, Investigation, Resources; Rone Cesar, Resources; Felipe Oliveira Costa, Investigation; Rafael Ponce-Terashima, Conceptualization; Luciano Kalabric Silva, Data Curation, Formal analysis, Writing – review & editing, Visualization; Mitermayer G. Reis, Conceptualization, Project administration, Resources, Supervision; Ronald E. Blanton, Conceptualization, Funding acquisition, Methodology, Formal analysis, Writing – review & editing; Lúcio M. Barbosa, Conceptualization, Investigation, Methodology, Writing – review & editing.

Data availability

Individual participant data collected during the study, after de-identification, will be available after publication to researchers who provide a methodologically sound proposal to achieve the aims in the approved proposal. Proposals should be directed to rblanton1@tulane.edu; to gain access, data requestors will need to sign a data access agreement. Dataset and study protocol will be available.

Competing Interests

The authors declare no conflicts of interest.

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Figures

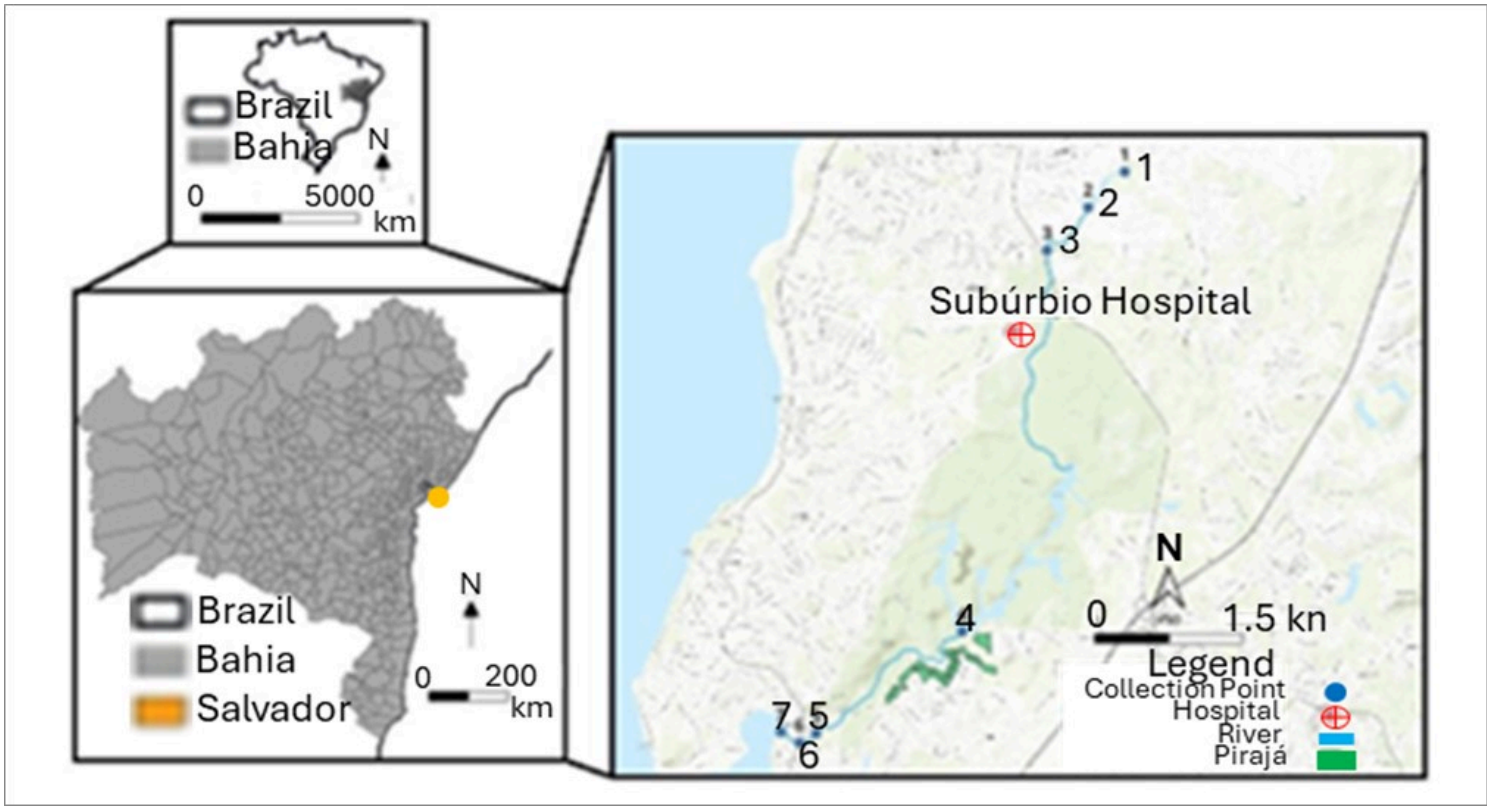


Figure 1

Microbiological collections in Salvador, Bahia, Brazil: Community, river, and hospital locations. The water collection points on the Cobre River are P1-7. The studied community area near Cobre river is represented in green. The hospital area is marked with a circled cross.

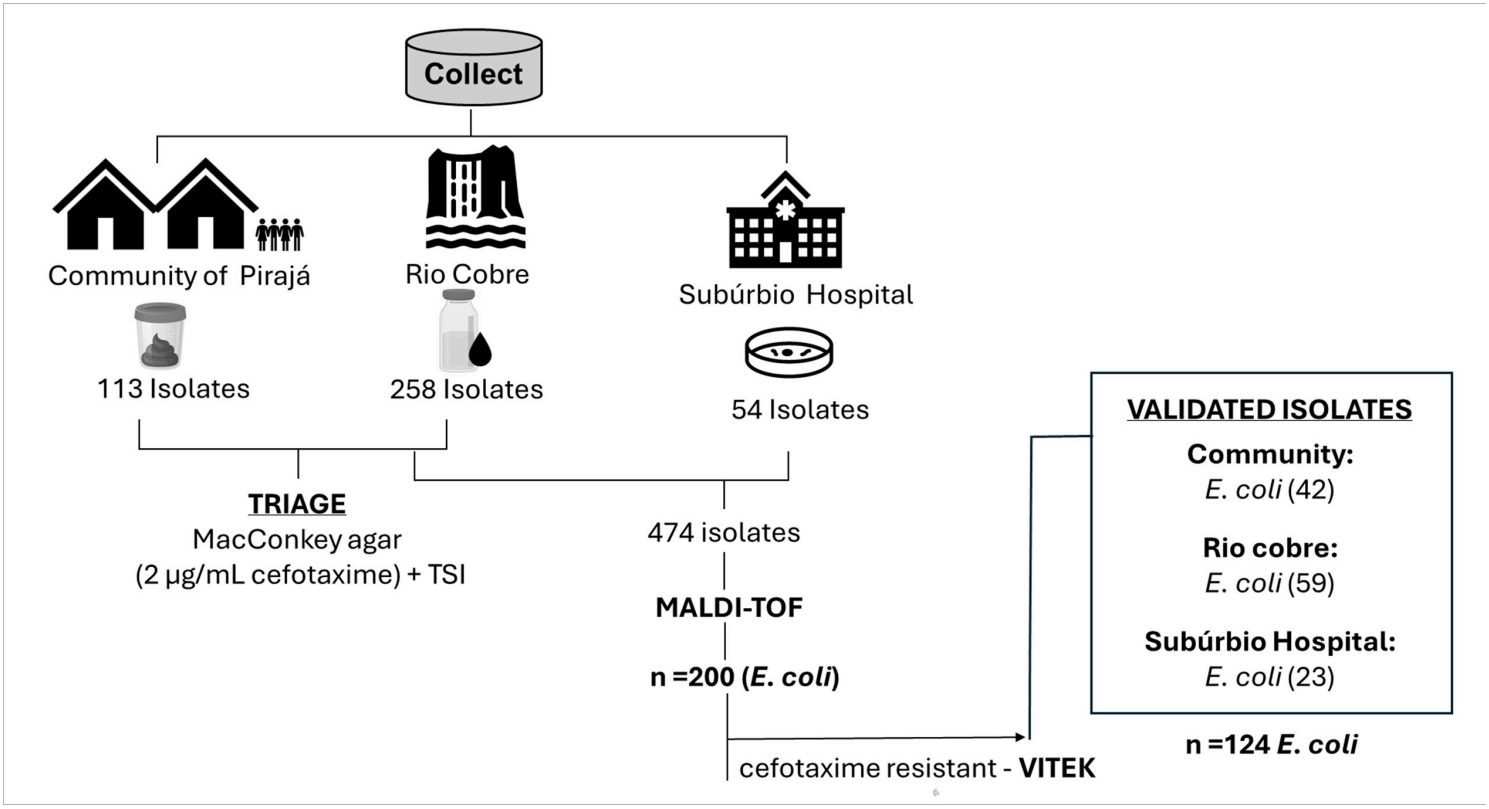


Figure 2

Study workflow: Fecal and water samples were triaged for *E. coli* resistant to 3rd generation cephalosporins on MacConkey agar and TSI medium with cefotaxime. Hospital collections were all *E. coli* from deep infections resistant to 3rd generation cephalosporins. All samples were confirmed by MALDI-TOF and VITEK-2 analysis.

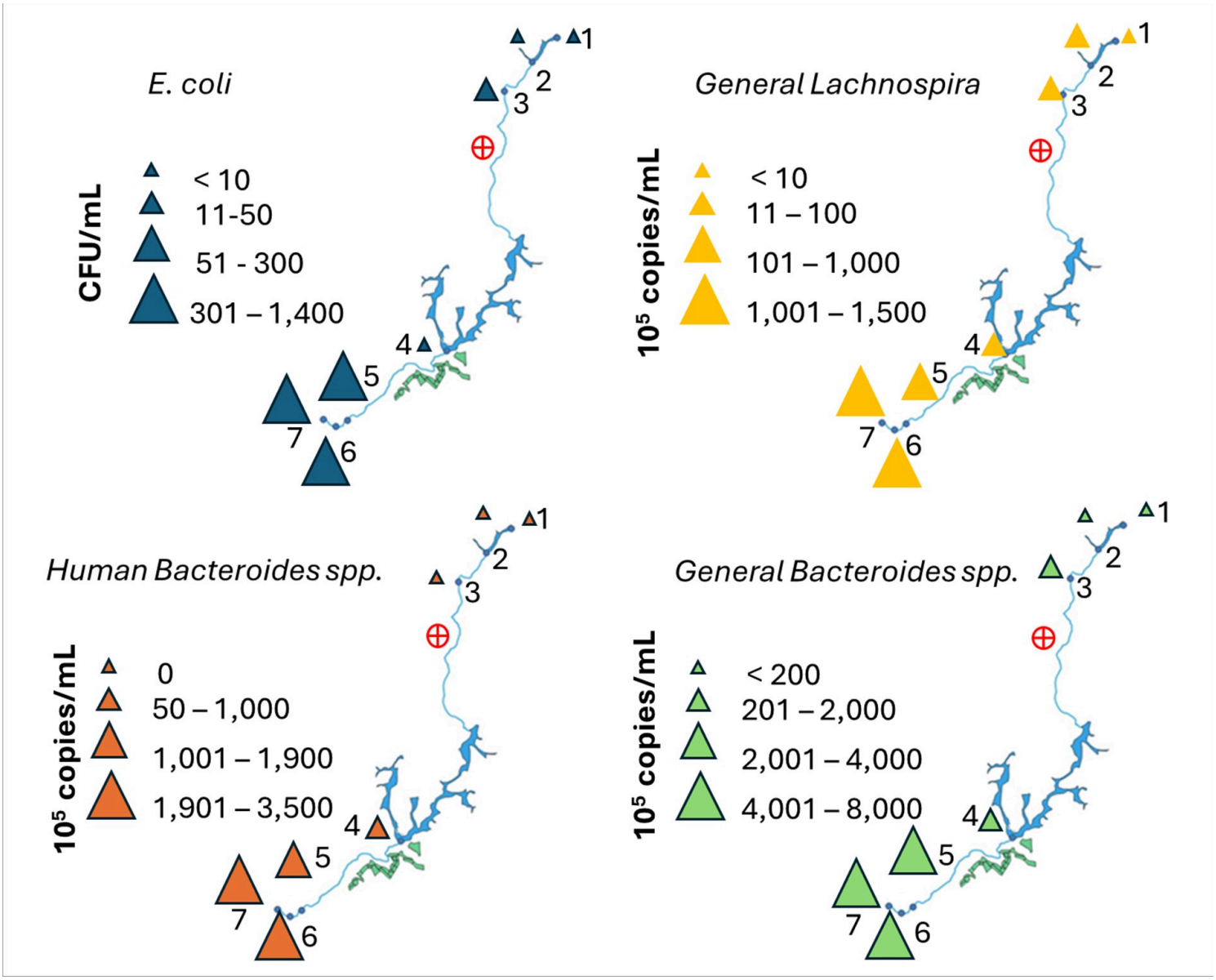


Figure 3

Tracking fecal contamination. Samples taken from points numbered 1-7 along the Cobre River in Salvador, Bahia, Brazil were tested quantitatively for *E. coli* growth and DNA copy numbers for *Bacteroides* and *Lacknospiras*pp. Both general and human indicative *Bacteroides* spp were assayed. The river flows from north to south through the park and the Cobre Reservoir to the mouth of the river and the highest population density. The Subúrbio Hospital is indicated by the circled cross and the segment of the Pirajá neighborhood sampled is indicated by the green section between point 4 and 5.

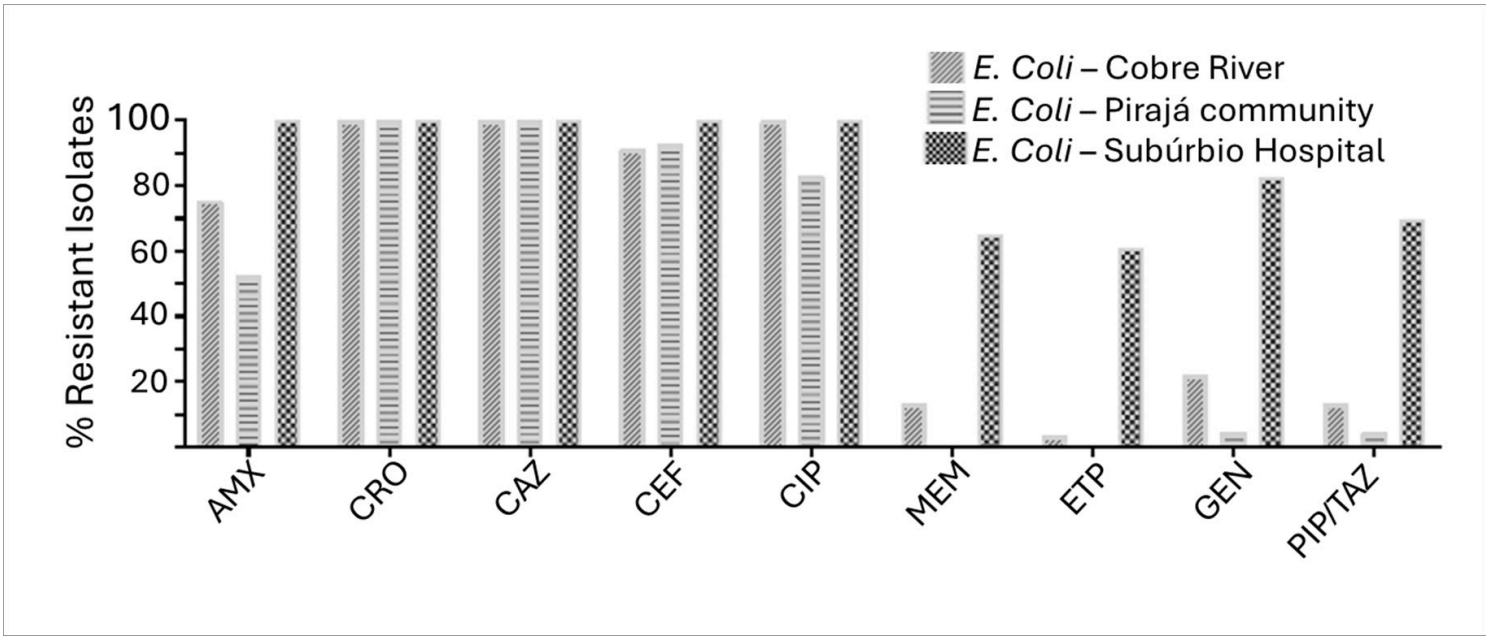


Figure 4

Susceptibility profile to antimicrobials tested among cefotaxime resistant *E. coli* isolates: Isolates from hospital infections more frequently showed resistance to other broad-spectrum antibiotics. AMX - amoxicillin/clavulanic acid, CRO - ceftriaxone, CAZ - ceftazidime, CEF - cefepime, CIP - ciprofloxacin, MEM - meropenem, ETP - ertapenem, GEN - gentamicin, PIP/TAZ - piperacillin/tazobactam

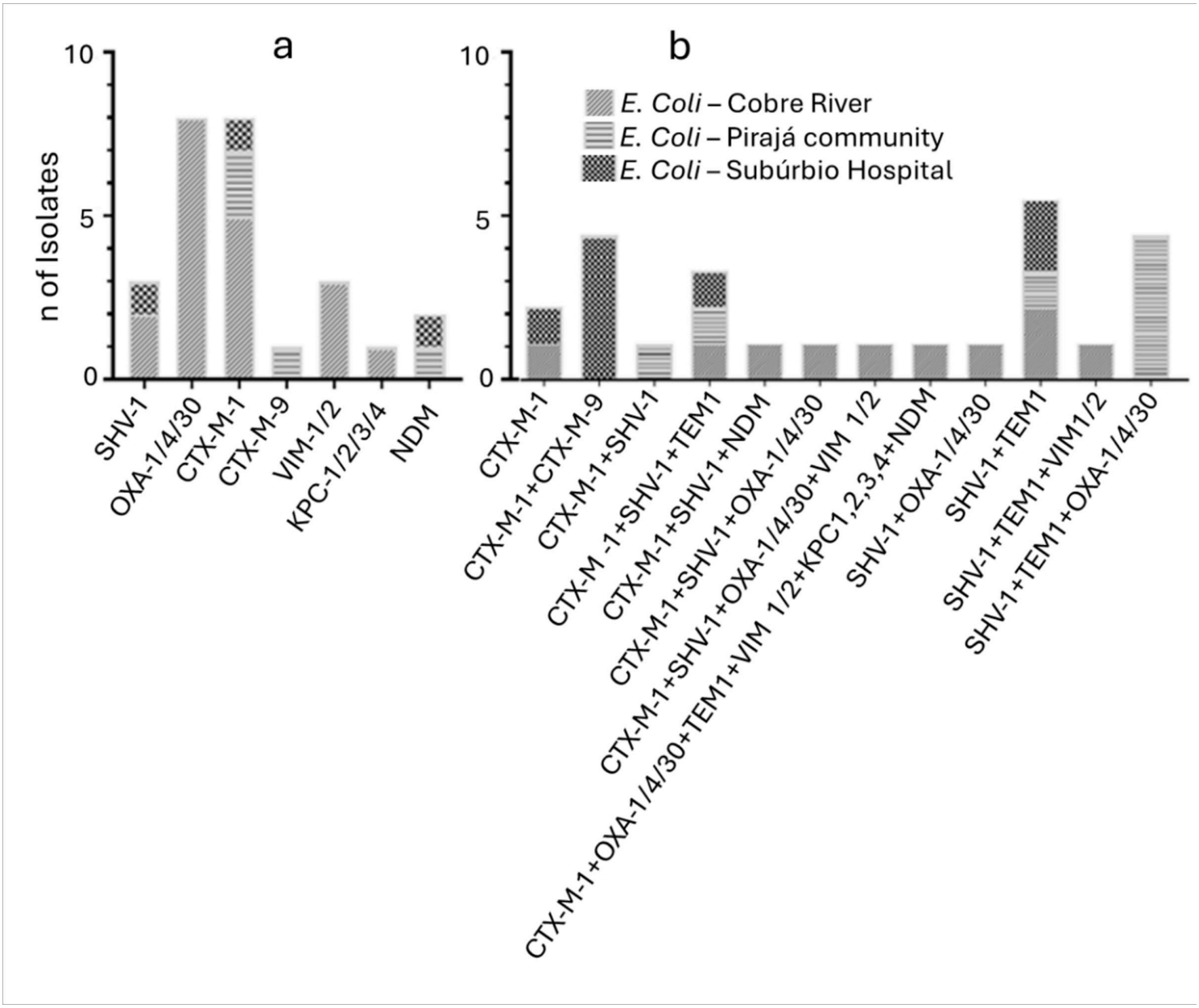


Figure 5

ESBL and Carbapenem resistance genes by compartment: Numbers of isolates presenting single (a) or multiple, (b) β-lactamase genes among *E. col*i isolates. Diversity of resistance genes was greatest in river samples.