Short Communication

Genomic features of a multidrug-resistant and mercury-tolerant environmental Escherichia coli recovered after a mining dam disaster in South America

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HIGHLIGHTS
• Genomic data of a MDR E. coli recovered after a mining dam disaster are presented.
• A broad resistome (antibiotics, hazardous heavy metals, and biocides) was predicted.
• E. coli sequence type ST219 carried the clinically relevant blaCTX-M-2 ESBL gene.
• The presence of the mer operon conferring mercury tolerance is highlighted.
• The role of impacted rivers in the spread of critical priority pathogens is discussed.

ABSTRACT
Mining dam disasters contribute to the contamination of aquatic environments, impacting associated ecosystems and wildlife. A multidrug-resistant Escherichia coli strain (B2C) was isolated from a river water sample in Brazil after the Mariana mining dam disaster. The genome was sequenced using the Illumina MiSeq platform, and de novo assembled using Unicycler. Resistome, virulome, and plasmidome were predicted using bioinformatics tools. Data analysis revealed that environmental E. coli B2C belonged to sequence type ST219 and phylogroup E. Strikingly, a broad resistome (antibiotics, hazardous heavy metals, and biocides) was predicted, including the presence of the clinically relevant blaCTX-M-2 extended-spectrum β-lactamase (ESBL) gene, qacEΔ1 efflux pump gene, and the mer (mercury resistance) operon. SNP-based analysis revealed that environmental E. coli B2C was clustered along to ESBL-negative E. coli strains of ST219 isolated between 1980 and 2021 from livestock in the United States of America. Acquisition of clinically relevant genes by ST219 seems to be a recent genetic event related to anthropogenic activities, where polluted water environments may contribute to its dissemination at the human-animal-environment interface. In addition, the presence of genes conferring resistance to heavy metals could be related to environmental pollution from mining activities. Antimicrobial resistance genes could be essential biomarkers of environmental exposure to human and mining pollution.

Keywords: Environmental pollution; Antimicrobial resistance; Genomic surveillance; Extended-spectrum β-lactamase; Mercury resistance; Critical pathogens; One health

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1. Introduction

Anthropogenic activities related to urbanization, industrialization, farming and animal food production have been responsible for introducing multidrug-resistant pathogens to aquatic environments in Latin America and worldwide (Dominguez et al., 2021). In this regard, although the World Health Organization (WHO) has deemed broad-spectrum cephalosporin-resistant and carbapenem-resistant Enterobacteriales as critical-priority pathogens (Tacconelli et al., 2018), the magnitude of the threat of antimicrobial-resistance from polluted aquatic environments has not been quantified. While production of extended-spectrum β-lactamases (ESBLs) has been the main mechanism for broad-spectrum cephalosporins, co-selection of antibiotic resistance due to heavy metal resistance is the mer system (mercury resistant operon), which consists of a mercury reductase, a lase, and other proteins (periplasmic, inner membrane, and regulatory) (Boyd and Barkay, 2012). Strikingly, heavy metals allow co-selection of antibiotic resistance due to co-resistance and cross-resistance mechanisms (Baker-Austin et al., 2006).

In fact, heavy metal-resistant E. coli producing ESBLs have been reported abroad (Azam et al., 2018; Sütterlin et al., 2014; Freitas et al., 2019; Yang et al., 2020).

Dam rupture constitutes a disruption of nature-mediated mass transfer between geological reservoirs on Earth, inducing severe environmental damage. Specifically, anomalous enrichment of trace metals found in aquatic environments evidence this damage (Buch et al., 2020), contributing to the selection of environmental microbiota expressing tolerance to hazardous heavy metals (Keim et al., 2021). In addition, contamination by dam disaster tailings has increased the amount and abundance of antimicrobial resistance genes in the environment (Furlan et al., 2020).

In 2015, Brazil experienced an environmental accident after an iron ore dam failure in the Doce River basin, Minas Gerais state (Carmo et al., 2017), which raised the local environmental contamination and the bioavailability of heavy metals (Aguiar et al., 2020). Although, the presence of antibiotic resistance genes in environments contaminated with heavy metal, as well as the role of heavy metals in co-selection and horizontal transfer of plasmid-mediated antibiotic resistance genes have been well documented (Yang et al., 2017; Chen et al., 2019; Imran et al., 2019; Zhang et al., 2018), there is a lack of data about selection of critical priority pathogens and/or presence of resistant determinants in long-term metal contaminated areas affected by environmental accidents. In this study, we have conducted a genomic and microbiologic investigation of WHO critical priority pathogens recovered from water samples collected from a river affected by a mining dam disaster.

2. Materials and methods

A local surveillance study was conducted to investigate the occurrence of critical priority bacteria along 84 km of the Doce River Basin (Minas Gerais State, southeastern Brazil), affected by a mining dam disaster. During 2018, samples were obtained from eight different sites comprising urban and rural areas. Briefly, 500 mL of surface water of each site were collected using sterile plastic bottles, and they were stored and transported to the laboratory at 4 °C. Samples were concentrated by filtering 100 mL of each sample using a 0.45 µm sterile membrane. To ensure maximum bacterial isolates, another 100 mL of water samples were centrifugated (5000 rpm/30 min). Filters and pellets were suspended in three milliliters of Brain Heart Infusion broth (Difco, USA) and incubated at 35 °C ± 2 °C for 24 h. Growths were used in the following steps.

2.1. Bacterial isolation, species identification and antibiotic susceptibility profile

Ten microliters of each broth with bacterial growth were streaked on MacConkey agar plates (Acumedia) supplemented with cetrioxone (2.0 µg/mL). Bacterial strains were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The antimicrobial susceptibility profile was determined by Kirby-Bauer method (CLSI, 2021), and ESBL production was screened using the double-disk synergy test (Rawat and Nair, 2010; CLSI, 2021).

2.2. Heavy metal tolerance

Tolerance to sodium arsenite (AsNa2O2; Baker & Adamson, USA), mercury chloride (HgCl2; Mallinckrodt, UK), copper sulfate (CuSO4.5H2O; Synth, Brazil), silver nitrate (AgNO3; Merck, Germany), cobalt chloride (CoCl2; Baker & Adamson, USA), and potassium dichromate (K2Cr2O7; Vetec, Brazil) was evaluated using a broth microdilution method (CLSI, 2021). Klebsiella pneumoniae strain KPN535 (One260, http://www.onehealthbr.com/), harboring mercury (merA-C), arsenic (araA-D, araH), copper (ccmA-A, ccmB-S), silver (sirR-S, sild, sib), and nickel (nkaA-E) resistance genes, and the E. coli ATCC strain 25922, negative for the presence of genes conferring tolerance to heavy metal tested, were used as controls.

2.3. Genomic analysis

DNA extraction was performed using the PureLink® Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) following the manufacturer’s instructions. DNA concentration was assessed by Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA). The genomic library was constructed using the Nextera XT DNA Library Preparation Kit (Illumina Inc., Cambridge, UK). The genome of the E. coli B2C strain was sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, CA) using 2 × 150 bp paired-end reads. Adaptors were removed using Trim Galore v.0.6.5 (https://github.com/FelixKrueger/TrimGalore). Then, filtering and trimming were performed using AfterQC v.0.9.7 (https://github.com/OpenGene/AfterQC) and Trimmomatic v.0.36, with the following parameters: LEADING = 20, TRAILING = 20, SLIDINGWINDOW = 4:20, HEADCROP = 10, and MINLEN = 85 (Bolger et al., 2014). Filtered and trimmed reads were de novo assembled using Unicycler v.1.2.10 (https://github.com/rrwick/Unicycler) (Wick et al., 2017). The draft genome sequence was automatically annotated using Rast: Rapid Annotation Using Subsystem Technology v. 2.0 server (https://rast.nmpdr.org) (Aziz et al., 2008) and Prokka (https://github.com/teemann/prokka) (Seemann, 2014). Genome size was calculated using Jellyfish v.2.3.0 (https://github.com/mgraciaJS/Jellyfish). Antimicrobial resistance genes were assessed using ResFinder v.4.0 (Zankari et al., 2012), Megasres v.2.0 (Doster et al., 2019), and Bacmac v.2.0 (https://bacman.biomedicine.gu.se) databases. Virulome, plasmidome, multilocus sequence typing (MLST), plasmid multilocus sequence typing (pMLST), fmH-type, and serotype were predicted using VFDB (Chen et al., 2016) and Ecoi_vf (https://github.com/phac-nml/ecoi_vf), PlasmidFinder (Carattoli et al., 2014), MLST 2.0, pMLST 0.1.0, FimTyper 1.0, and SeroTypeFinder 2.0.1, respectively, available at the Center for Genomic Epidemiology (https://genomicepidemiology.org/). Prediction of contigs as plasmid-derived was performed using milplasmids v.3.4.1 tool (https://github.com/sirarredondo/milplasmids) (Arredondo-Alonso et al., 2018). Phylogroup was determined in silico using the ClermonTyping tool (http://clermonotyping.iame-researccheer.org/) (Beghain et al., 2018). A ≥ 95% identity threshold was used to identify all genes.

2.4. Phylogenetic analysis

Raw reads of E. coli were submitted to the Escherichia/Shigella database in Enterobase (https://enterobase.warwick.ac.uk). The genome assembly of E. coli strain B2C (Enterobase Ubereinstr: ESC_WA0085AA) was downloaded from Enterobase, along with all available genome assemblies from ST219 lineages, which had data for source of isolation, country, and year of collection. A total of 34 E. coli ST219 assemblies were downloaded. The B2C genome was compared to each genome of the same ST to assess the average nucleotide identity (ANI), using FastANI v1.32 (https://github.com/ParBLiSS/FastANI). All downloaded genomes of E. coli ST219 were used with B2C for phylogenetic tree construction. CSI Phylogeny v1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny) was used with default settings to generate a SNP-based maximum-likelihood phylogenetic
gene associated with disinfectant resistance (Tong et al., 2021), being flanked upstream by an IS911-type insertion sequence element (Fig. 1). In this regard, disinfectants and heavy metals are co-selecting substances that contribute to the spread of antibiotic resistance genes (Baker-Austin et al., 2006; Pal et al., 2015).

For B2C strain, tolerance to mercury chloride (MIC, 8 μg/mL) was three times greater than for E. coli ATCC 25922 (MIC, 1 μg/mL), and identical (MIC, 8 μg/mL) to the one shown by the mer operon-positive K. pneumoniae KP535 strain (Table 2). In this respect, heavy metal resistance genes for mercury (merACDTPR), arsenic (arsRBC), nickel (nkhABCDE), copper (pcaABCDR, cauBCFRS, cuEOR, cuA), zinc (znuABC, znuAR, znuB, znuR), and silver (sulABCEFP5) were identified in E. coli B2C strain. The presence of several heavy metal resistance genes in E. coli strain B2C seems unusual compared to other E. coli strains recovered from the environment (Azam et al., 2018; Siddiqui et al., 2020). However, this difference could be due to the use of whole-genome sequencing, whereas most studies describe the detection of a few selected genes using PCR alone. Therefore, our finding could give more evidence about the role of environmental areas contaminated with heavy metal in the selection of bacterial isolates acquiring antimicrobial resistance genes.

Whole-genome sequence analysis generated 2,290,538 paired-end reads assembled in 139 contigs, with 107× coverage, N50 value of 159,706, and a G + C content of 50.62%. The B2C strain presented a genome size of 5,391,093 bp, containing 82 rRNAs and six rRNAs. Circos plot (Zrywinski et al., 2009) and subsystem annotation obtained from the RAST server are shown in Fig. 2A and B, respectively.

Virulone analysis identified salmochelin (inoN), colicin V (cvaC), type 1 fimbrin (fimH), outer membrane hemoglobin receptor (chaU), hemolysin E (hlyE), heat-stable enterotoxin (astA), and E. coli common pilus (ecpA-E) genes.

The E. coli B2C strain also harbored a plasmid of incompatibility group IncFIB (belonging to FAB formula F24-A: B1), which has already been detected in E. coli strain harboring the blaCTX-M-2 gene from seagulls (Laurus dominicanus) in Argentina (Liakopoulos et al., 2016). The IncF F24-A: B1 has also been referred to as a successful plasmid with wide dissemination among pathogenic or commensal E. coli strains (Liakopoulos et al., 2016).

Although, in this study, was not possible to obtain the complete nucleotide sequence of plasmids due to limitations of short-read methodology, analysis using mplasmids v.2.1.0 (https://sarrerdono.shinypaps.io/mplasmids) showed that the ESBL-encoding blaCTX-M-2 gene and the mercury resistance operon (merRTPCADE) were carried by the IncF [F24-A: B1] plasmid, along with genes encoding resistance to tetracycline (tetA), sulfonamides (sul1), aminoglycosides [aac(3)-Vla] and quaternary ammonium efflux pump (qacEAl). Additionally, the blaCTX-M-2 gene was found to be located upstream of an IS911 insertion sequence (Fig. 1), being both flanked by duplicated copies of sul1-qacEAl genes that may have resulted from an insertion sequence event (Poirel et al., 2008).

Furthermore, blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of the pB2C plasmid partial sequence (33.7 kb in length) showed that it was highly similar to an IncF [F16-A-B-] plasmid identified in Salmonella enterica serovar Kentucky isolated from chicken wing in the United States of America (Genbank accession number CP082700), sharing 99.99% nucleotide pair-wise identity and 82% query coverage. This finding denote how these genes have been disseminated in aquatic environments, highlighting the possibility of gene transfer from/to other species (Chen et al., 2019; Fu et al., 2019).

The B2C strain was assigned to phylogroup E, commonly associated with bovine lineages (Coura et al., 2015a; Coura et al., 2015b). In this regard, the Doce river basin has dairy farms along its shore, and most cows have free access to the river, where feces can reach the watercourse contributing to the bacterial load.
all isolates was 84.80%, corresponding to 4,382,433 positions found in all analyzed genomes. Single-nucleotide polymorphisms (SNPs) count among all 34 genomes of ST219 analyzed ranged between 0 and 5,273 SNPs, when using default settings (Minimum distance between SNPs of 10 bp) (Supplementary material 1). Disabling minimum distance between SNPs raised maximum SNP distance among the genomes to 10,163; but raising the distance to 100 bp reduced maximum SNP distance to 1,076. However, the resulting trees had similar clusters to the one generated with default settings. High differences in SNPs values highlight the degree of genetic variation among ST219 strains, with relatively distant strains within the same ST. However, the low number of available ST219 genomes on Enterobase may also interfere in this result. Finally, it is important to point out that contigs were used for the phylogenetic analysis instead of reads. Therefore, the minimum distance between SNPs (pruning) was the only possible adjustment in CSI Phylogeny because the other parameters are related to the quality of reads.

The phylogenetic tree clustered the B2C strain with five ESBL-negative E. coli ST219 strains isolated between 1980 and 2021, from livestock and poultry, in the United States of America (USA) (Fig. 3). Besides to the phylogroup E assignment, the phylogenetic clustering supports the hypothesis that E. coli B2C can be an animal (mostly livestock)-derived lineage.

Anthropogenic activities (e.g., industrial, household, hospital, and agricultural) are responsible for releasing large volumes of waste in the watercourses and contribute to the spread of antimicrobial-resistant bacteria (Squadrone, 2020). The presence of antibiotic- and heavy metal-resistant bacteria in aquatic environments can further contribute to the dissemination of antibiotic resistance genes to associated ecosystems (Gomi et al., 2017). In fact, a previous metagenome investigation on bacterial microbiota and resistomes in cows from dairy farms at the Doce River basin region revealed the presence of Enterobacteriales and ESBL genes (Gaeta et al., 2020). Therefore, critical priority pathogens can achieve animal and human host throughout water and contaminated food crops (Verrae et al., 2013; Onicicu et al., 2019).

A particular concern about heavy metals and antibiotic resistance is that metals are not biodegradable and involve long-term selective pressure (Song et al., 2017). Heavy metals are agents that contribute to the indirect selection of antibiotic resistance genes, mainly by co-selection of cross-resistance (Pal et al., 2018). The co-selection is essential to disseminate and maintain antibiotic resistance even in pristine environments, where no or few antibiotics are used (Pal et al., 2018). Cross-resistance occurs when a single system, such as an efflux pump, confers resistance to different determinants (e.g., disinfectants, antibiotics, and heavy metals) (Chapman, 2003; Baker-Austin et al., 2006). On the other hand, co-resistance occurs when distinct antimicrobial resistance genes are physically linked to the same genetic element (e.g., transposon, plasmid). Consequently, resistance to one antimicrobial compound results in simultaneous resistance to others (Baker-Austin et al., 2006).

The presence of different resistance genes within the same genetic context has a particular concern regarding horizontal gene transfer when a conjugative plasmid is involved (Rasmussen and Sorensen, 1998). In this regard, heavy metals seem to facilitate the conjugative transfer in environmental bacteria. Indeed, copper and zinc were found to accelerate the conjugative transfer of antimicrobial resistance genes in freshwater bacteria (Wang et al., 2020; Wang et al., 2021), whereas E. coli strains co-harboring antibiotic and heavy metal resistance (e.g., mer operon and/or sulEPS, merBPT, and arsC) genes have been described in contaminated aquatic environments (Azam et al., 2018; Chen et al., 2019; Sultan et al., 2020).

Our findings corroborate the genetic linkage between mer operon and antibiotic resistance genes in bacterial isolates found in polluted aquatic environments (Mcintosh et al., 2008). Strikingly, in the E. coli B2C strain, the mercury resistance operon and antibiotic and disinfectant resistance genes were located on the same mobile genetic element (i.e., IncF plasmid). Thus, our results reinforced the evidence about the correlation between antibiotic resistance and mine-related contamination, and suggest the occurrence of both the co-resistance mechanism and horizontal gene transfer among bacteria in the Doce River basin.

Lately, the environmental co-selection of resistance to cephalosporins and tetracyclines by selective mercury pressure has been demonstrated in Bacillus spp. isolates from a mining district in Almadén, Spain (Robas et al., 2021). Thus, our results reinforced the evidence about the correlation between antibiotic resistance and mine-related contamination.

Dam rupture constitutes a severe disruption resulting in a severe and significant mass transfer between geological reservoirs on Earth, inducing environmental damage visible in microbiota, flora, fauna, soils, and water. For instance, we know that 60 Mt. of mining waste from the Mariana dam was released into Rio Doce ecosystems on November 5, 2015. For comparison, a transfer rate of 1.6 Gt/year of continental debris has been quantified for the West African region, which means ~43 Mt./day of matter transfer (Grimaud et al., 2018). A similar tectonic context shared by West Africa and Brazil supports that analogy. The massive quantity of iron tailings from Mariana dam implies a transfer of several trace metals (i.e., Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn) leading to bioaccumulation of x3.2 times than those attained in a laboratory for Hg (Buch et al., 2020). The bioaccumulation of metals in the environment incontestably causes

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**Table 2**

Minimum inhibitory concentrations (µg/mL) of heavy metals for E. coli B2C, and E. coli ATCC 25922 and K. pneumoniae KPN535 control strains.

| Heavy-metal          | Minimum inhibitory concentration (µg/mL) | E. coli B2C | E. coli ATCC 25922 | K. pneumoniae KPN535 |
|----------------------|------------------------------------------|-------------|-------------------|----------------------|
| Arsenic (AsNO3)      | 512                                      | 32          | > 1024            |
| Cobalt (CoCl2)       | 1024                                     | 512         | 512               |
| Copper (CuSO4.5H2O)  | 1024                                     | 1024        | 2048              |
| Chromium (K2Cr2O7)   | 256                                      | 128         | 128               |
| Mercury (HgCl2)      | 8                                        | 1           | 8                 |
| Silver (AgNO3)       | 2                                        | 4           | 4                 |
adverse effects on biological processes and may contribute to the selection and dissemination of antimicrobial resistance genes (Baker-Austin et al., 2006; Buch et al., 2020). Indeed, a significant correlation between heavy metal bioaccumulation in fish muscle and the occurrence of heavy metal resistance genes in *E. coli* has been described (Ture et al., 2020).

Consequently, iron tailings also imply an anomalous enrichment of trace metals becoming toxic for several species. In addition, a dramatic consequence is time-related, given that residence time for Hg is around 20 to 30 years in oceanic waters (Gworek et al., 2016). Therefore, it is highly advisable to monitor the consequences of this dam rupture on Brazilian marine waters.

Data on the background levels of antimicrobials in the Doce River basin’s water and/or sediments are scarce. Approximately 60 Mt. of mining tailings were responsible for increasing the concentration of some metals. Copper, nickel, and zinc were higher in the reducible sediment fractions and associated with the tailings’ original composition (Aguiar et al., 2020). A recent study comparing trace metals in sediments before and after the disaster showed that the mud was the source of cadmium, and arsenic was present before the environmental disaster. However, its concentration increased due to sediment remobilization (Daarte et al., 2021).

The Samarco Company refers that the mud did not contain a dangerous concentration of heavy metals, and it was composed mainly of silt (47.5%),
Fig. 3. In A, phylogenetic tree of 34 Escherichia coli strains belonging to ST219. Genome sequences and epidemiological information (i.e., sources of isolation, predicted antimicrobial resistance phenotype, country, and year of collection) were downloaded from Enterobase (www.enterobase.warwick.ac.uk). ISO 3166-1 Alpha-2 country codes: BR, Brazil; CA, Canada; DE, Germany; DK, Denmark; EE, Estonia; GB, United Kingdom; IN, India; JP, Japan; KE, Kenya; NL, Netherlands; QA, Qatar; SE, Sweden; TW, Taiwan; US, United States. In B, a subtree of the highlighted cluster shows the resistome and plasmidome of the isolates.
followed by fine sand (37.5%), clay (10.6%), and coarse sand (4.5%). However, independent studies such as those conducted by the Minas Gerais State Water Agency (IGAM) detected high levels of mercury, arsenic, cadmium, copper, chromium, lead, zinc and nickel in water and/or sediments samples from the Doce River following the rupture (IGAM, 2022), suggesting that the ore tailings may also have minor and trace elements, as found in the Brumadinho slurry (Vergilio et al., 2020).

Copper, nickel, and aluminum were above the maximum permissible value by Brazilian legislation in the water 2 years after de accident (Carvalho et al., 2017). Indeed, results from a water and sediment quality assessment in the coastal zone around the mouth of Doce River after the accident indicated that the dam rupture affected water and sediment quality in the Atlantic Ocean but also showed that the concentrations of the toxic elements are returning slowly to the levels before the accident (Richard et al., 2020). Finally, water samples used in the present study were also evaluated by wavelength dispersive X-ray fluorescence (data not published). The mean concentration of aluminum (3.79 mg/L), copper (0.2 mg/L), and iron (15.4 mg/L) were above the Brazilian regulations (CONAMA. National Environment Council, 2005), while mercury, cobalt, and arsenic were not detected. Therefore, even though the long history of pollution of the Doce River basin (domestic and mining effluents containing toxic elements), the mining tailings spill may have potentiated the heavy metal contamination in the river (Richard et al., 2020; Santana et al., 2021), and increased the selective pressure on bacteria, regarding antimicrobial resistance genes.

In summary, we report genomic and microbiological data of an environmental E. coli belonging to ST219, co-harboring the clinically relevant blaCTX-M-3 ESBL gene and mer operon genes conferring tolerance to mercury (a hazardous waste problem); recovered from a Brazilian river impacted by a mining dam disaster. Our results suggest that the acquisition of clinically relevant resistance genes by the environmental E. coli ST219 seems to be a genetic event related to anthropogenic activities. In contrast, the presence of genes conferring resistance to heavy metals could be related to environmental pollution from mining activities. Therefore, antimicrobial and heavy metal resistance genes could be essential biomarkers of environmental exposure to human and mining pollution.

Nucleotide sequence accession number

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JACSWD0000000000. The version described in this paper is version JACSWD0000000000.1 (Bio Project PRJNA658122). Genomic information of E. coli B2C strain is available on the OneBR platform under the number ID ONE113 (http://onehealthbr.com/).

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CRediT authorship contribution statement

Natália C. Gaeta: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. Daniel U. de Carvalho: Investigation. Herrison Fontana: Investigation, Formal analysis. Quêzia Moura: Investigation. Bruna Fuga: Investigation. Patricio Montecinos Munoz: Writing – review & editing. Lilian Gregory: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. Nilton Lincopan: Conceptualization, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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