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METAGENÔMICA APLICADA AO ESTUDO DE ÁGUA DOCE DESTINADA AO CONSUMO HUMANO NO MUNICÍPIO DE OURO PRETO, MINAS GERAIS

Departamento de Microbiologia
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Tese apresentada ao Programa de Pós-graduação em Microbiologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do grau de Doutor em Microbiologia.

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“O sonho encheu a
noite
“Extravasou pro meu dia
Encheu minha vida
E é dele que eu vou viver
Porque sonho não morre.”

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SUMÁRIO

RESUMO

Águas doces superficiais e subterrâneas são ecossistemas aquáticos importantes, que abrigam uma diversidade microbiana complexa e são frequentemente utilizadas como fonte de água para consumo humano. Sendo assim, o estudo do microbioma dessas águas é relevante, uma vez que está diretamente relacionado com saúde pública. Metagenômica, em especial, sequenciamento de alto rendimento do rDNA 16S, tem sido empregado para investigar a comunidade procariótica de fontes de água potável, permitindo um conhecimento mais amplo da estrutura e dinâmica microbiana autóctone do ambiente. Considerando a relevância do tema, o presente trabalho teve como objetivo determinar a composição taxonômica e a diversidade da comunidade de Bacteria e Archaea de fontes de água subterrânea e superficial usadas para consumo humano e a dinâmica da comunidade no sistema de tratamento de água potável do município de Ouro Preto, Minas Gerais. As amostras de água coletadas foram filtradas (poro de 0,22 µm) e a extração de DNA foi realizada empregando-se *kit* comercial, a partir das membranas filtrantes. O sequenciamento da região V4 do rDNA 16S foi conduzido em plataforma *Illumina MiSeq*. As sequências geradas foram processadas e, então, agrupadas em OTUs (97% de identidade). As fontes de água superficial exibiram diversidade e composição procariótica similares, sem variações sazonais. Contudo, as fontes de água subterrânea demonstraram um aumento significativo da diversidade na estação chuvosa e diferença na composição procariótica entre as estações. O filo Proteobacteria, classe Betaproteobacteria, OTU *Comamonadaceae* não classificada, predominou nas fontes de água doce usadas para abastecimento alternativo e público, com exceção das águas subterrâneas na seca, nas quais Firmicutes, classe Bacilli, particularmente *Bacillus* e *Enterococcus*, estiveram presentes em abundância superior. No que se refere a características físico-químicas, observou-se, principalmente, correlação positiva significativa dos táxons Bacteroidia, Clostridia, Gammaproteobacteria e Flavobacteria com parâmetros que indicam poluição orgânica e de Alphaproteobacteria, Betaproteobacteria e Cytophagia com parâmetros indicativos de qualidade adequada da água. Além disso, várias OTUs estavam positivamente correlacionadas com metais pesados, dentre elas *Acinetobacter*, *Bacillus* e *Enterococcus*. Bactérias potencialmente patogênicas relevantes, como *Acinetobacter baumanni* e *Escherichia coli*, foram detectadas nas fontes usadas para abastecimento público e alternativo, o que impõe um risco potencial para a população, em especial, quando a água é consumida sem tratamento. O tratamento da água alterou a estrutura da comunidade, observando-se a mudança do predomínio do filo Proteobacteria, classe Betaproteobacteria, na água bruta para Firmicutes, classe Bacilli, OTUs *Geobacillus*, *Bacillaceae* não classificada, *Enterococcus*, Bacillales não classificada e *Paenibacillus*, na água pós-tratada e no ponto final de distribuição. A redução dos índices de diversidade microbiana e da contagem do rDNA 16S confirmou o efeito do tratamento sobre a comunidade procariótica da água bruta. Resultado preocupante foi a detecção de bactérias potencialmente patogênicas no sistema, como *Enterococcus faecalis*, *Mycobacterium intracellulare* e *A. baumannii*, indicando a resistência dessas bactérias ao tratamento e persistência das mesmas no sistema de distribuição de água.

Palavras-chave: comunidade procariótica, água para consumo, tratamento de água.

ABSTRACT

Surface freshwater and groundwater are important aquatic ecosystems that harbor complex microbial diversity and are often used as drinking water. Therefore, the study of the microbiome of these waters is relevant, because it is directly related to public health. Metagenomics, particularly 16S rDNA sequencing has been employed to investigate the procaryotic community of drinking water sources, promoting a better understanding of the structure and autochthonous procaryotic dynamics of the environment. Considering the relevance of the subject this study aimed to determine the taxonomic composition and diversity of the procaryotic community of groundwater and surface water sources used for human consumption and community dynamics in the drinking water treatment system of the municipality of Ouro Preto, Brazil. Water samples were filtered (0.22 µm pore size) and DNA was extracted from the membranes using a commercial kit. Sequencing of the V4 region of the 16S rDNA was performed by Illumina Miseq and the generated sequences were processed and clustered into OTUs (97% identity). Surface water sources exhibited similar procaryotic diversity and composition showing no seasonality. However, groundwater sources showed a significant increase in diversity in the rainy season and differences in procaryotic composition between seasons. The phylum Proteobacteria, class Betaproteobacteria, OTU *Comamonadaceae* not classified, predominated in freshwater sources used for alternative and public supply, except for groundwater in the dry season in which Firmicutes, class Bacilli, particularly *Bacillus* and *Enterococcus*, were detected in a higher abundance. In regard to physicochemical characteristics, a significant positive correlation was observed between Bacteroidia, Clostridia, Gammaproteobacteria, and Flavobacteria taxa with parameters that indicate organic pollution and Alphaproteobacteria, Betaproteobacteria, and Cytophagia with parameters that indicate suitable water. In addition, several OTUs were significantly correlated with heavy metals, among them *Acinetobacter*, *Bacillus*, and *Enterococcus*. Relevant potentially pathogenic bacteria such as *Acinetobacter baumannii* and *Escherichia coli* were detected in water sources used for public and alternative supply, representing a potential risk to the population, especially for those not submitted to treatment. Water treatment changed community structure. While phylum Proteobacteria, class Betaproteobacteria, predominated in raw water, phylum Firmicutes, class Bacilli, *Geobacillus*, *Bacillaceae* unclassified, *Enterococcus*, Bacillales unclassified, and *Paenibacillus* OTUs were dominant in treated water samples (post-treated and tap water). Reduction of procaryotic diversity indexes and 16S rDNA count confirms the effect of treatment on the microbial community of raw water. The detection of potentially pathogenic bacteria such as *Enterococcus faecalis*, *Mycobacterium intracellulare* and *A. baumannii* throughout the distribution system, indicating their persistence consequently to the resistance to treatment processes raises particular concern.

Keywords: procaryotic community, drinking water, water treatment.

1 INTRODUÇÃO

1.1 PROBLEMÁTICA DA ÁGUA

A água é essencial em todos os segmentos da vida, sendo considerada um recurso insubstituível. A oferta de água para o abastecimento tem sido apontada como um dos grandes problemas do século XXI, ressaltando-se que a abundância do elemento líquido causa uma falsa sensação de recurso inesgotável. Entretanto, 97,5% da água disponível na Terra é salgada, sendo imprópria para o consumo humano. Apenas 2,5% da água é doce, 2,493% inacessível em geleiras ou regiões subterrâneas (aquíferos) e somente 0,007% é encontrada em rios, lagos e na atmosfera (WHO, 2011).

As atividades humanas, respaldadas em um estilo de vida e desenvolvimento, têm determinado alterações significativas no meio ambiente, influenciando na disponibilidade e qualidade da água. Os desmatamentos crescentes, os processos de erosão/assoreamento dos mananciais superficiais e os lançamentos de efluentes e detritos industriais e domésticos nos recursos hídricos têm contribuído para tal situação (CANN *et al.*, 2013). Sendo assim, as sociedades enfrentam um enorme desafio para o manejo de recursos hídricos e para a provisão de água potável (TRAM *et al.*, 2014).

Esta problemática ainda é agravada pelas disparidades de acesso da população ao abastecimento de água e a serviços sanitários básicos entre países desenvolvidos e em desenvolvimento e entre regiões dos próprios países. Por exemplo, o Brasil possui a maior disponibilidade hídrica do mundo, com um potencial de água doce extremamente favorável para os diversos usos. No entanto, sua distribuição ocorre de forma irregular entre as regiões. No Norte, onde habita apenas 7% da população, estão concentrados, aproximadamente, 70% do total da água disponível para uso, enquanto os 30% restantes distribuem-se desigualmente pelo País, para atender a 93% da população. A região Nordeste, onde vive cerca de 28% da população brasileira, dispõe de apenas 3% dos recursos hídricos nacionais. Além da distribuição geográfica heterogênea e inadequada, atualmente, muitas cidades e zonas rurais não possuem sistemas de tratamento de água, em torno de 16% abastece-se de água proveniente de fontes inseguras e, quando

atendidos por rede pública, nem sempre recebem água com qualidade adequada e suficiente (SNIS, 2015).

Há uma relação clara entre o saneamento adequado e a melhoria da qualidade de vida e da saúde da população. Entretanto, o Brasil ainda apresenta um *deficit* histórico em termos de cobertura de serviços básicos de saneamento. Nos últimos anos, ocorreram avanços significativos dos investimentos em saneamento, sobretudo do acesso da população aos serviços de água potável, mas, o mesmo não foi verificado para a oferta de serviços de esgotamento sanitário. No Brasil, apenas 42,6% dos esgotos produzidos passam por algum processo de tratamento antes de serem descartados no ambiente e mais de 3,5 milhões de brasileiros, nas 100 maiores cidades do País, despejam esgoto irregularmente, mesmo tendo redes coletoras disponíveis, revelando o problema crônico da falta de saneamento básico no País (SNIS, 2015).

Um exemplo interessante da problemática da água no Brasil é o município de Ouro Preto, situado no estado de Minas Gerais. Ouro Preto é uma cidade turística que possui Serviço Municipal de Água e Esgoto (SEMAE/OP), porém, insuficiente para o atendimento de toda a demanda populacional. Assim, devido à riqueza de diversas fontes de água para consumo na região, a população utiliza águas alternativas, incluindo outros mananciais e nascentes, e fontes subterrâneas, como poços tubulares e antigas minas de ouro abandonadas, para abastecimento doméstico, público e institucional (FONSECA; PRADO, 2006).

1.2 ÁGUA PARA CONSUMO HUMANO

A qualidade necessária à água distribuída para consumo humano é a potabilidade, ou seja, a água deve ser tratada, limpa e estar de acordo com um conjunto de parâmetros estabelecidos por normas e legislações sanitárias em relação aos níveis microbiológicos, químicos, físicos ou radioativos, não devendo, em hipótese alguma, oferecer riscos à saúde humana (MS, 2011). Essa potabilidade é alcançada mediante várias formas de tratamento, sendo que a mais tradicional inclui, basicamente, etapas de coagulação, floculação, decantação, filtração, desinfecção e fluoretação (WHO, 2011).

Para atender a este padrão, a água para abastecimento deve apresentar quantidades limite para diversos parâmetros físico-químicos e microbiológicos, que são definidos pela portaria nº 2.914 (MS, 2011). A avaliação da qualidade microbiológica tem um papel de destaque no processo, em vista do número elevado e da grande diversidade de microrganismos com reconhecido potencial patogênico, muitas vezes, de origem fecal, que podem estar presentes na água (PREST *et al.*, 2016).

"O ser humano bebe 80% de suas doenças". Este é o provérbio médico citado por Bouguerra (2004), quando discute a água como carreadora de diversos patógenos, sendo, seu controle, uma questão de saúde pública. Dados revelam que milhões de pessoas, principalmente crianças, morrem anualmente por doenças relacionadas à água, em todo o mundo (WHO, 2016). Além disso, cerca de 80% das doenças que ocorrem em países em desenvolvimento são veiculadas pela água contaminada com microrganismos (WHO, 2016).

As principais doenças relacionadas à ingestão de água contaminada comumente relatadas são as de transmissão fecal-oral, como cólera, febre tifoide, hepatite A, doença diarreica aguda e poliomielite. Contudo, existem outros patógenos menos comuns, mas, também, veiculados pela água, que merecem atenção (WHO, 2011). Além dos patógenos de transmissão fecal-oral, há diversos patógenos ambientais, muitas vezes oportunistas, que podem permanecer viáveis na água potável e nos sistemas de distribuição (ASHBOLT, 2015a).

As doenças entéricas estão associadas a patógenos transmitidos pelas fezes. Entre os mais importantes, estão aqueles que causam doença diarreica, como as bactérias *Escherichia coli*, *Salmonella enterica*, *Shigella*, *Campylobacter jejuni* e *Vibrio cholerae* (WHO, 2011). As doenças oportunistas, geralmente, não estão relacionadas à ingestão de água ou transmissão fecal-oral, mas, a água é um reservatório dos agentes etiológicos das mesmas (ALLEN *et al*., 2013). Diversas espécies bacterianas Gram negativas clinicamente importantes ocorrem, naturalmente, em ambientes aquáticos e são bem adaptadas a colonizar os sistemas de distribuição de água, que incluem tubulações, reservatórios e caixas d'águas, principalmente formando biofilmes. Como exemplo, podem ser citadas *Legionella, Pseudomonas aeruginosa, Acinetobacter baumannii* e *Mycobacterium avium* (FALKINHAM, 2015b).

Os métodos aplicados na rotina para a avaliação da qualidade microbiológica de águas para consumo humano são os que detectam e enumeram os indicadores fecais. Este termo “indicador” se refere a um determinado microrganismo ou grupo de microrganismos que evidencia poluição da água analisada com material de origem fecal, proveniente de seres humanos ou de outros animais, demonstrando a possibilidade da presença de algum patógeno entérico na amostra (SAXENA *et al.*, 2015). Coliformes, principalmente, *Escherichia coli*, têm sido extensivamente utilizados no monitoramento da qualidade de águas e são considerados os indicadores de qualidade de água para avaliação de potabilidade e balneabilidade mais específicos (WHO, 2011).

A pesquisa de coliformes é o principal método de avaliação de contaminação em água potável em todo mundo. Alguns outros indicadores também são reconhecidos como adequados, como *Enterococcus* e bacteriófagos. Ainda, há sugestões adicionais, como *Bacteroides*, *Clostridium perfringens*, *Bifidobacterium* (SAXENA *et al.*, 2015), *Blautia* e *Prevotella* (MCLELLAN; EREN, 2014), ou até mesmo componentes orgânicos fecais produzidos pela microbiota indígena intestinal, como esteróis (ZHENG *et al.*, 2014).

Contudo, diversas pesquisas têm revelado a limitação dos indicadores tradicionais, em especial, das bactérias do grupo coliforme, pois não se correlacionam necessariamente com a presença de outros patógenos importantes que, frequentemente, são observados em água, como vírus (RIGOTTO, 2009), bactérias oportunistas, como *Legionella* spp., *Mycobacterium* spp. e *Pseudomonas aeruginosa*, e protozoários (WANG *et al.,* 2012).

Desta forma, nas últimas décadas, o uso de métodos de genética molecular para identificar rapidamente, em água potável, patógenos que infectam seres humanos, vem sendo considerado como uma abordagem promissora de saúde pública. Desde a década de 1990, vários projetos que envolvem essas técnicas para detecção de vírus, protozoários e bactérias na água potável, como a detecção de *Enterococcus*, *Bacteroides* e vírus entéricos em águas recreativas (ASHBOLT, 2015a), foram financiados. Reação de polimerização em cadeia quantitativa (qPCR) empregando, como alvo, o rDNA 16S de *Enterococcus* foi o primeiro método de genética molecular aprovado pela Agência de Proteção Ambiental dos Estados Unidos (EPA) para avaliação de água recreativa impactada por esgoto e água tratada. Este ensaio, que detecta enterococos totais, viáveis ou não, fornece

o melhor índice de risco para a saúde após exposição à água fresca e marinha em estudos epidemiológicos (HAUGLAND *et al.*, 2014).

Mais recentemente, avanços importantes no uso desse grupo de métodos foram observados, em especial, a possibilidade do emprego do sequenciamento de nova geração. Os custos de sequenciamento estão diminuindo tão rapidamente que abordagens metagenômicas de gene alvo, como o rDNA 16S, vêem promovendo a detecção rápida e mais ampla de patógenos em amostras clínicas e ambientais, constituindo-se em ferramentas epidemiológicas muito utilizadas (ASHBOLT, 2015b). Com base nesses estudos, a expectativa é de que muitos patógenos importantes de água potável atualmente não cultiváveis serão identificados nas próximas décadas, além dos patógenos clássicos. Nos Estados Unidos, por exemplo, já se relatou que, em 45% dos surtos associados à água potável, não são identificados os agentes etiológicos, em parte, devido à incapacidade de cultivá-los (CRAUN *et al.*, 2010). Mesmo alguns patógenos bacterianos bem conhecidos perdem a capacidade de cultivo quando transportados do intestino para o ambiente aquático, como, por exemplo, *Campylobacter* (LI *et al.*, 2014).

1.3 METAGENÔMICA

A metagenômica define-se como o estudo do metagenoma, que é o material genético obtido diretamente de comunidades microbianas específicas de um determinado *habitat* (HANDELSMAN, 2004). Os estudos metagenômicos iniciaram-se no final da década de 80, primeiramente, com a proposta de extrair DNA diretamente de amostras ambientais (PACE *et al.*, 1985). O termo metagenômica, porém, foi proposto somente em 1998, por Handelsman e colaboradores, a partir da sugestão de uma série de procedimentos para acessar o metabolismo de microrganismos desconhecidos no solo (HANDELSMAN *et al.*, 1998). O seu estabelecimento revolucionou o estudo dos microrganismos e de seu impacto na ecologia, na indústria, na agricultura e em diversas outras áreas e representou um enorme avanço na compreensão da diversidade biológica e, ainda, no entendimento da evolução de patógenos e na disseminação de genes associados

à resistência a antimicrobianos. Por fim, é fundamental para a descoberta de novos genes, proteínas e vias metabólicas (QUINCE *et al.*, 2017).

Em termos práticos, existem diversas abordagens que compõem o que se chama atualmente de metagenômica (FORBES *et al.*, 2017). Com o desenvolvimento e aprimoramento dos sequenciadores de primeira geração (baseados no método de Sanger) para os de Nova Geração (do inglês *Next Generation Sequencing* ou, simplesmente, NGS, como a plataforma Roche 454, Ion-Torrent e, principalmente, o Illumina MiSeq, duas abordagens distintas surgiram e são comumente usadas: a primeira é referida como metagenômica direcionada e a segunda denomina-se metagenômica *shotgun* (DUDHAGARA *et al.*, 2015). Na metagenômica *shotgun*, sequencia-se o DNA total do ambiente. Em contrapartida, na metagenômica direcionada, várias regiões do genoma podem ser selecionadas para o sequenciamento, incluindo genes funcionais, mas, as opções mais populares envolvem o uso de genes conservados, como rDNA 16S, rDNA 18S e ITS, que fornecem uma melhor resolução na identificação da estrutura taxonômica de uma comunidade microbiana (OULAS *et al.*, 2015).

Estudos de comunidades microbiana utilizando NGS permitiram uma melhor compreensão do microbioma de água doce, criando novos conceitos biológicos (GERBER, 2014). Porém, até poucos anos atrás, estes estudos estavam focados em espécies únicas e limitados às circunstâncias artificiais dos meios de cultura, sem um contexto ambiental (SLEATOR *et al.*, 2008).

1.4 MICROBIOMA DE ÁGUA DOCE

A água, como elemento fundamental da natureza viva, abriga inúmeros organismos, representados por algas, protozoários, fungos, bactérias e vírus. A água doce está entre os *habitats* naturais que abrigam a diversidade microbiana mais rica (TAMAMES *et al.*, 2010). Num estudo comparativo envolvendo vários *habitats* naturais e artificiais, Tamames *et al.* (2010) concluíram que o solo e a água doce, incluindo aquíferos, águas subterrâneas, lagos, rios, água potável e águas para consumo, são os *habitats* naturais que abrigam o maior e o mais diverso grupo bacteriano.

As bactérias são componentes biológicos importantes nesse ecossistema e desempenham papel fundamental na ciclagem de nutrientes essenciais, como a decomposição de compostos orgânicos (TAYLOR *et al.*, 2010) e a produção primária nas cadeias alimentares aquáticas (RIEMANN, 1985). A importância biogeoquímica das bactérias nos ecossistemas de água doce foi reconhecida, pela primeira vez, na década de 1940, quando Lindeman (1942) descreveu a dinâmica trófica de um lago temperado do norte. Desde então, tornou-se claro que as bactérias aquáticas impulsionam as transformações e a ciclagem da maioria dos elementos biologicamente ativos nesses ecossistemas. Esta posição-chave, ocupada em, praticamente, todos os ciclos biogeoquímicos, não se deve apenas ao papel das bactérias como principais degradadores de compostos orgânicos, mas também resulta da sua produção de biomassa, que alimenta a rede alimentar e tem um profundo impacto na qualidade da água no ecossistema (NEWTON *et al.*, 2011).

Interessantemente, menos de 0,1% dessas bactérias podem ser cultivadas e são, habitualmente, autóctones da água. Exemplos de grupos quase exclusivamente ou exclusivamente detectados por métodos independentes de cultivo são Deltaproteobacteria, Epsilonproteobacteria, Acidobacteria, Verrucomicrobia, Cyanobacteria, Nitrospirae, Planctomycetes, Chloroflexi, Chlorobi, Gemmatimonadetes, Spirochaetes, Chlamydiae, Aquificae, Thermotogae, Fusobacteria, Synergistetes e Tenericutes (VAZ-MOREIRA *et al.*, 2014).

No que se refere às categorias taxonômicas superiores, em geral, as bactérias predominantes em água doce pertencem aos filos Proteobacteria (principalmente das classes Alphaproteobacteria, Betaproteobacteria e Gammaproteobacteria), Actinobacteria, Bacteroidetes e Firmicutes, independentemente do tipo de água, como rios, água mineral, água potável e águas residuais. No entanto, observa-se uma especificidade aparente para alguns tipos de água. Por exemplo, os membros da classe Betaproteobacteria e do filo Bacteroidetes são, frequentemente, detectados na superfície, água mineral e água potável, mas não tão frequentemente em águas residuais. Por sua vez, Firmicutes são, comumente, relatados em águas residuais. Inversamente, diferentes tipos de água apresentam padrões distintos de diversidade bacteriana em categorias taxonômicas inferiores, como gênero e espécie (SIMON; DANIEL, 2011; VAZ-MOREIRA *et al.*, 2014).

Em relação a gêneros, vários são detectados em diferentes tipos de *habitats* aquáticos, como águas residuais, superficiais e água potável, como *Acidovorax*, *Curvibacter*, *Sphingomonas*, *Aeromonas*, *Acinetobacter*, *Pseudomonas*, *Legionella*, *Rhodococcus*, *Gordonia*, *Mycobacterium*, *Flavobacterium*, *Bacillus* e *Clostridium*. As bactérias incluídas nestes grupos e outras ainda não identificadas são, provavelmente, capazes de circular entre diferentes *habitats* aquáticos, abrangendo também todo o ciclo urbano da água (MCLELLAN *et al.*, 2015).

Em contraste, as arqueias, geralmente, são encontradas em abundância menor em ecossistemas aquáticos dulcícolas. Entretanto, estudos metagenômicos atuais estão revelando uma alta diversidade e um papel fundamental de arqueias nos ciclos biogeoquímicos desses ambientes, como o ciclo do nitrogênio, no qual realizam várias etapas, a oxidação de enxofre e o ciclo do carbono, na decomposição da matéria orgânica (AUGUET *et al.*, 2010).

O uso de técnicas independentes de cultivo revolucionou a maneira de se estudar as arqueias e tem trazido um conhecimento mais refinado e abrangente, alterando sua antiga classificação taxonômica. Vários filos atuais estão sendo criados, incluindo Thaumarchaeota e Odinarchaeota, além daqueles que incluem espécies cultiváveis, como Euryarchaeota e Crenarchaeota. Por isso, os bancos de dados atuais ainda apresentam defasagem quanto à classificação taxonômica desses microrganismos (AGUIAR-PULIDO *et al.*, 2016).

1.5 METAGENÔMICA APLICADA AO ESTUDO DE ÁGUA DOCE DESTINADA AO CONSUMO HUMANO

Por muitas décadas, os microbiologistas confiaram, principalmente, nos métodos de cultivo padrão, direcionados a organismos indicadores, para avaliar a diversidade microbiana dos ecossistemas naturais e, mais especificamente, para monitorar a qualidade microbiana geral da água potável (MACHADO *et al.*, 2014). Entretanto, estima-se que mais de 99% dos microrganismos não são cultiváveis ou são de difícil cultivo por técnicas de rotina (BRETTAR; HOFLE, 2008), incluindo muitos patógenos transmitidos pela água, por exemplo, *Vibrio cholerae*, *Mycobacterium* e *Legionella*, que podem permanecer em estado viável, mas não cultivável (VEZZULLI *et al.*, 2013). Assim, o monitoramento da água potável

seguindo os padrões internacionais de qualidade não garante, necessariamente, a boa qualidade para o consumidor (BURTSCHER *et al.*, 2009). Atualmente, vários estudos são conduzidos usando abordagens metagenômicas direcionadas ao rDNA16S e *shotgun*, para caracterizar a composição de comunidades procarióticas e a ecologia geral em ecossistemas de água doce destinadas ao consumo humano (LYMPEROPOULOU *et al.*, 2012; MACHADO; BORDALO, 2014; ZHANG *et al.*, 2014; LIU *et al.*, 2016; MUKHERJEE *et al.*, 2016; FARENHORST *et al.*, 2017).

Distúrbios ambientais ou atividades humanas podem levar a perturbações no microbioma das fontes de água doce, incluindo mudanças no perfil de microrganismos endógenos e introdução de microbiota fecal de origem humana ou animal. Essas mudanças na estrutura da comunidade em combinação com parâmetros ambientais podem apontar para a fonte de perturbação na qualidade da água. Assim, um melhor entendimento de todo o microbioma das águas doces e de suas fontes de poluição é crítico para avaliar as mudanças na comunidade microbiana e as ameaças associadas ao ecossistema e à saúde humana. Estudos utilizando abordagens metagenômicas permitem a detecção dessas perturbações e a identificação de biomarcadores para detecção de poluição (UYAGUARI-DIAZ *et al.*, 2016).

1.6 METAGENÔMICA APLICADA AO ESTUDO DE SISTEMA DE TRATAMENTO DE ÁGUA DESTINADA AO CONSUMO HUMANO

O tratamento de água produz água potável, removendo os contaminantes físicos, químicos e microbiológicos presentes, em atendimento aos regulamentos de saúde pública, por meio de vários processos de tratamento, geralmente, incluindo coagulação, sedimentação, filtração e desinfecção. As estações de tratamento e os sistemas de distribuição atuam como barreiras protetoras, produzindo água potável, sendo monitorados para evitar a contaminação e a multiplicação de microrganismos, principalmente daqueles que causam doenças, ao longo do processo até o destino final (LIU *et al.*, 2017).

Atualmente, várias pesquisas têm sido realizadas em sistemas de tratamento de água para consumo humano utilizando NGS, com o intuito de ampliar o conhecimento ecológico das comunidades microbianas do sistema, revelando uma

grande diversidade de comunidades bacterianas e eucarióticas, bem como mudanças substanciais na comunidade após as etapas de tratamento (GOMEZ-ALVAREZ *et al.*, 2012; PINTO *et al.*, 2012; CHAO *et al.*, 2013; LAUTENSCHLAGER *et al.*, 2013; SHI *et al.*, 2013; HUANG *et al.*, 2014; LIN *et al.*, 2014; WANG *et al.*, 2014; DOUTORELO *et al.*, 2016; STANISH *et al.*, 2016; LI *et al.*, 2017; XU *et al.*, 2017; SALEEM *et al.*, 2018).

Estudos empregando NGS demonstram que a composição microbiana qualitativa e quantitativa varia espacial e temporalmente nos sistemas de água potável. A fonte de água doce bruta captada introduz, principalmente, as comunidades bacterianas presentes nos sistemas de tratamento e tem efeito significativo nas comunidades bacterianas do sistema (WU *et al.*, 2015; LI *et al.*, 2017). Em geral, os dois primeiros tratamentos aplicados à fonte de água são floculação e sedimentação, que não alteram significativamente a estrutura da comunidade microbiana (LIN *et al.*, 2014). A filtração limita a influência da fonte de água na comunidade bacteriana do sistema de distribuição, porque é projetada para remover turbidez e substratos para multiplicação bacteriana e depende de estruturas comunitárias estáveis (PINTO *et al.*, 2012). A desinfecção, etapa final do tratamento da água, geralmente realizada utilizando cloro, é fundamental para controlar o microbioma liberado na água tratada e controlar a multiplicação microbiana durante a distribuição (LI *et al.*, 2017). No entanto, a desinfecção, que objetiva controlar, e não eliminar, o microbioma em sistemas de tratamento, pode atuar, potencialmente, como pressão para seleção de amostras resistentes (GOMEZ-ALVAREZ *et al.*, 2012; CHAO *et al.*, 2013; HUANG *et al.*, 2014), com ênfase em potenciais patógenos, como *P. aeruginosa*, *Mycobacterium* spp., *Acinetobacter baumanni* e *Leptospira interrogans* que, classicamente, são conhecidos por persistirem em água da torneira (HUANG *et al.*, 2014).

2 JUSTIFICATIVA

A água potável é uma necessidade básica para o desenvolvimento humano, o bem-estar e a saúde de qualquer sociedade. Entretanto, a água limpa e segura nem sempre é acessível ou está disponível. Quando a qualidade microbiológica da água fica comprometida, ela torna-se veículo de transmissão de vários microrganismos, em especial, associados à doença diarreica aguda, e de outros patógenos oportunistas. Desta forma, a avaliação da qualidade da água veiculada para o consumo se mostra importante e imprescindível para a proteção da saúde pública. Atualmente, a qualidade microbiológica da água é avaliada pela presença de bactérias indicadoras, como os coliformes. Entretanto, esta abordagem demonstra muitas falhas, principalmente, por ter uma abrangência de detecção limitada, dependente do cultivo dos microrganismos. Recentemente, técnicas de genética molecular avançadas, como a metagenômica, utilizando sequenciadores de nova geração, têm sido utilizadas para uma análise mais representativa de toda a comunidade microbiana autóctone de cada ambiente e para a detecção de patógenos que circulam no ambiente. Estas técnicas têm mostrado grandes vantagens, como rapidez e abrangência dos resultados e fornecimento de informações úteis para a melhoria da gestão das águas para consumo humano, mas, ainda não estão sendo aplicadas em estudos de água para consumo no Brasil. Desta forma, parece-nos pertinente a utilização do sequenciamento de alto rendimento para investigar o perfil da comunidade microbiana de amostras de água destinada ao consumo humano no País. Nossa área de estudo é a cidade de Ouro Preto, cuja população dispõe de diferentes fontes superficiais e subterrâneas de água para o consumo, usadas para abastecimento alternativo e oficial, que, historicamente, exibem problemas quanto à qualidade microbiológica. Este estudo visa a fornecer dados ecológicos microbianos da água de Ouro Preto e seu possível impacto na saúde pública.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Determinar o perfil da comunidade procariótica (domínios Bacteria e Archaea) da água para consumo do município de Ouro Preto, Minas Gerais, Brasil.

3.2 OBJETIVOS ESPECÍFICOS

- Determinar a dinâmica e a diversidade da comunidade de Bacteria e Archaea ao longo do sistema de tratamento e abastecimento público de Ouro Preto (respondido no artigo 1).
- Caracterizar a estrutura e a diversidade das comunidades de Bacteria e Archaea de fontes de água doce superficial e subterrânea usadas para consumo humano de Ouro Preto (respondido no artigo 2).
- Avaliar a influência das propriedades físico-químicas nas comunidades de Bacteria e Archaea das fontes de água doce superficial e subterrânea usadas para consumo humano de Ouro Preto (respondido no artigo 2).
- Investigar a presença de bactérias de transmissão hídrica reconhecidas como potencialmente patogênicas nas fontes de água doce superficial e subterrânea usadas para consumo humano e ao longo do sistema de tratamento e abastecimento público de Ouro Preto (respondido nos artigos 1 e 2).

4 ARTIGOS CIENTÍFICOS

4.1 ARTIGO 1

ARTIGO A SER SUBMETIDO AO PERIÓDICO *WATER RESEARCH*

Bacterial community dynamic analysis evidentiates the persistence of Firmicutes in a drinking water treatment system

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Highlights

- Drinking water treatment benefited the dominance and persistence of the Firmicutes.
- Drinking water treatment decreased diversity and bacterial count.
- An increased abundance of pathogenic bacteria throughout the DWTS is a cause of concern.

Abstract

16S rRNA gene deep sequencing has been used to investigate bacterial community composition and diversity of a drinking water treatment system (DWTS), increasing the knowledge of the microbial dynamics characteristic of the environment. However such a kind of approach had never been used to evaluate DWTS in Brazil. The aim of this study was to investigate the dynamics of the structure and diversity of the bacterial community through a DWTS fed from surface water flowing through rocks in Ouro Preto, an important touristic city located in southeastern Brazil. Water samples were collected in the rainy and dry seasons from three different points as follows: raw water (RW), post-treated water (PTW), and tap water (TW) and evaluated by 16S rRNA gene deep sequencing. Dominant bacterial taxa at the highest taxonomic levels (phylum and class) were changed by water treatment, shifting from Proteobacteria, class Betaproteobacteria in RW to Firmicutes, class Bacilli in PTW until the end-point distribution (TW). Within the class Bacilli, the OTUs of *Geobacillus* and unclassified *Bacillaceae*, in the rainy season and *Enterococcus*, unclassified Bacillales and *Paenibacillus* in the dry season predominated in treated water samples. Bacterial diversity and 16S rRNA gene counts decreased consequently to water treatment. Potentially pathogenic bacteria such as *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium intracellulare*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were detected in DWTS what should be considered as an alarming result from a health point of view. Data generated add knowledge to the understanding of the microbiological

effects of DWTS, highlighting the importance of Gram-positive Firmicutes resistance to treatment processes and their dominance in treated water.

Keywords: drinking water treatment system, bacterial 16S rRNA gene sequencing, water bacterial community, Firmicutes.

1 Introduction

A drinking water treatment system (DWTS) consists in several processes, including flocculation, sedimentation, filtration, and disinfection, designed to promote decontamination of raw water (RW) and distribution of safe drinking water (DW) to the population. The microbiological safety of treated water is particularly important for public health considering the need for eliminating potential pathogens frequently associated to the high prevalent so-called waterborne diseases (Prest et al., 2016; Li et al., 2017). Thus, better knowledge about microbial taxonomic composition and diversity in RW and treated water may allow the improvement of water treatment and distribution processes (Prest et al., 2016).

Advanced molecular genetic technologies, such as metagenomics, particularly 16S rRNA gene deep sequencing have been used in several current studies on DWTS (Hwang et al., 2012; Pinto et al., 2012; Pinto et al., 2014; Ling et al., 2016; Li et al, 2017). These studies reveal the complexity of DW microbiome and the persistence of microbial communities in full-scale DWTS (McCoy and VanBriesen, 2014; Pinto et al., 2014). Shifts in bacterial community structure and diversity consequently to water treatment have been observed in several systems. Such shifts may result from different chlorine resistance profile expressed by each bacterial group resulting in selective removal of some members of the bacterial community from RW (Hwang et al., 2012; Chao et al., 2013; Hou et al., 2018).

Gram-negative bacteria, particularly the phylum Proteobacteria, classes Alpha-, Beta-, and Gammaproteobacteria frequently show persistence throughout DWTS (Vaz-Moreira et al.,

2017; Saleem et al., 2018) and many of these treatment resistant bacteria detected at taps are potential pathogens, e.g. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Proctor and Hammes, 2015; Prest et al., 2016). On the other hand, there are very few reports on Gram-positive bacteria such as Firmicutes in treated water and most studies reveal them as a less abundant group (Pinto et al., 2012; Vaz-Moreira et al., 2013; Liu et al., 2016; Vaz-Moreira et al., 2017).

In regard to water origin, most studies aimed at evaluating the impact of treatment processes on bacterial communities (Pinto et al., 2012; Roeselers et al., 2015) focus on DWTS that use lake (Sallem el al., 2018) or river (Chao et al., 2013; Lin et al., 2014) as sources of RW, while other complex environmental surface water sources have been minimally explored, impairing knowledge about DWTS bacterial communities. In this context, it has already been shown that microbial dynamics are probably influenced by the characteristics of RW (Hammes et al., 2010; Henne et al., 2013; Sun et al., 2014), in addition to the classically discussed factors such as treatment process, pipe material, and disinfectant type (Prest et al., 2016).

In this study, the bacterial community diversity and taxonomic composition in a DWTS in Ouro Preto, a touristic city located in southeastern Brazil was investigated by 16S rRNA gene deep sequencing during the rainy and dry seasons. In this water supply network, RW is collected from a differentiated surface water source which is water flowing through rocks and treatment processes include coagulation, sedimentation, filtration, and disinfection. We aimed at investigating the dynamics of bacterial diversity and community composition variations and identifying persistent bacterial taxa and potentially pathogenic bacteria throughout the DWTS, specially at the point of use.

2 Materials and methods

2.1 Drinking water treatment system

Water samples were obtained from the public DWTS Itacolomi, located in Ouro Preto city, Minas Gerais, Brazil (20.23°S, 43.30°W). The facility is operated by the Municipal Water and Sewage Service (SEMAE), which captures RW flowing through rocks originated from Itacolomi State Park. The water treatment consists of coagulation/rapid hydraulic mixing (Parshall flume); Alabama type flocculator, divided into two parallel lines; two conventional decanters in parallel; five self-cleaning down filters; and a contact tank, where sodium hypochlorite is added to the water before leaving the DWTS. The mean flow rate is of 86 to 92 L/s.

2.2 Sampling and physiochemical analysis

RW, post-treated water (PTW) and tap water (TW) were sampled in the months of February and August, 2016 corresponding to rainy and dry seasons (rainfall rates 166 mm and 17 mm, respectively; www.climatempo.com.br/ouropreto). TW was collected from the main hospital of Ouro Preto. Samples were filtered using 0.22 µm nitrocellulose membranes (Millipore, USA) up to membrane saturation and they were stored at -80 °C until DNA extraction. Water filtered volumes varied from 2.0 L (raw water) to 6.5 L (treated water). Temperature, pH, dissolved oxygen (DO), and total dissolved solids (TDS) were measured in all samples using the HQ40D Portable Multi Meter (Hach, USA). Free chlorine was analyzed using the DR 2800 portable spectrophotometer (Hach, USA) and turbidity was determined using the 21100Q Portable Turbidimeter (Hach, USA). All measures were monitored in situ. Total solids (TS), nitrate, nitrite, ammonia, phosphate, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) were analyzed as recommended by the Standard Methods for the Examination of Water and Wastewater (Apha, 2012).

2.3 DNA extraction, library construction and sequencing

Total DNA was extracted with the aid of E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA), according to the manufacturer's instructions, and quantified with a Qubit fluorometer (Thermo Fisher Scientific, USA). 16S rRNA gene amplicon analysis was carried out using paired-end libraries with the universal primer pair (515F: 5'-GTGYCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACNVGGGTWTCTAAT-3') targeting the V4 hypervariable region of Bacteria (Caporaso et al., 2012; Klindworth et al., 2013). The library construction was performed following the protocol provided by Illumina for 16S rRNA gene library construction (16S Metagenomic Sequencing Library Preparation, 2013). Sequencing was performed on a MiSeq platform (Illumina, USA), using the MiSeq Reagent Kit v2 (500 cycles).

2.4 Sequence and statistical analysis

Raw reads were processed following Mothur MiSeq SOP available at Mothur website (Kozich et al., 2013) using Mothur software package version 1.37.5 (Schloss et al., 2009). Processing included removal of low quality (< 20), singleton, and chimeric sequences according to the Uchime algorithm (Edgar et al., 2011). Clean reads were aligned, classified against Silva v.123 16S rRNA gene database, and clustered into operational taxonomic units (OTUs) with a similarity cutoff of 97%. Alpha diversity (observed OTUs, Chao1 estimator, and Shannon index) and beta diversity indices were assessed using the R packages Phyloseq and Vegan (McMurdie and Holmes, 2013; Oksanen et al., 2016) based on rarefying the number of reads. Unweighted UniFrac distances (Lozupone et al., 2010) were determined for each sample separately (RW, PTW, and TW in the rainy and dry seasons) and ordinated by principal coordinate analysis (PcoA). Venn diagrams were constructed with “gplots” R package (Warnes et al., 2016). The affiliation of potential pathogens at the species level was analyzed through a BLASTn search in PathoSystems Resource Integration Center (PATRIC) 16S rRNA gene database with 100% of sequence coverage and identity.

2.5 Real-time PCR (qPCR) for bacterial quantification

Total bacterial load was determined by qPCR using the StepOnePlus™ Real-Time PCR System (Life Technologies, USA), employing universal primers for 16S rRNA gene (338F: TACGGGAGGCAGCAG; 518R: ATTACCGCGGCTGCTGG) according to Muyzer et al. (1993). All reactions were performed in triplicate. Specific amplification was confirmed by checking the melt curve of the target gene for each sample. A standard curve was prepared from a 10-fold serial dilution of the pool of all water samples DNA, which were amplified using conventional PCR and the same primers were employed for qPCR (R^2 > 0.99, efficiency = 90%).

3 Results

3.1 Drinking water physicochemical characteristics

Physicochemical characteristics of RW and treated water (PTW and TW) are shown in Table 1. Temperature increased through the DWTS as follows: from 19.5 in RW to 24.5 and 24.8 in PTW and TW in the rainy season, and from 18.5 in RW to 21.5 and 22.5 in PTW and TW in the dry season, respectively. The values of turbidity, TS, and COD were higher in RW samples obtained in the rainy season (1.32, 0.05, and 75.0, respectively) when compared to those collected in the dry season (1.00, 0.02, and 1.35, respectively). Lower values of these parameters were observed in treated water samples (PTW and TW) when compared to RW in both seasons. The values of pH were higher in all water samples obtained in the rainy season (7.7 for RW, 8.6 for PTW, and 7.7 for TW, respectively) than in those collected in the dry season (5.5 for RW, 5.9 for PTW, and 6.9 for TW, respectively). The values of free chlorine were higher in PTW samples (2.5 and 1.2, for dry and rainy seasons, respectively) and decreased in TW samples (0.4 and 1.0, for dry and rainy

seasons, respectively) in both seasons. The other parameters such as TDS, DO, BOD, nitrate, nitrite, and phosphate showed less variation between RW, PTW, and TW samples.

3.2 Overview of 16S rRNA gene-based sequencing

Sequencing generated a total of 731,912 raw reads (161,361 and 146,911 for RW, 101,316 and 110,515 for PTW, and 111,411 and 100,398 for TW, in the rainy and dry seasons, respectively). After assembly of contigs, processing, and filtering, 409,603 quality-checked reads (average length 253 bp) were retrieved (106,625 and 99,367 for RW, 59,361 and 47,902 for PTW, and 57,233 and 39,115, in the rainy and dry seasons, respectively).

3.3 Dynamics of DWTS bacterial community

Distinct microbial composition patterns between RW and treated water samples (PTW and TW) were observed. On the other hand, PTW and TW bacterial communities were highly similar, particularly in the dry season (Fig. 1; 2A, B).

In regard to RW samples (the following results presented refer to rainy and dry seasons, respectively), Proteobacteria were dominant in both seasons (64.7% and 47.4%), followed by Bacteroidetes (7.0% and 37.0%), Firmicutes (4.0% and 7.0%), Cyanobacteria (4.0% and 4.0%), Planctomycetes (1.0% and 4.0%), and Actinobacteria (0.5% and 1.6%) (Fig. 1). The most prevalent Proteobacteria class was Betaproteobacteria (39.0% and 35.0%) and the OTU more representative of this class was unclassified *Comamonadaceae*, corresponding to 32.0% and 24.0% of the total reads (Fig. 2A, B). Alphaproteobacteria (13.0% and 5.0%) composed mainly by the OTU unclassified Rhizobiales (2.3% and 0.8%) was also abundant while Gammaproteobacteria (3.0% and 7.0%) was less abundant. Interestingly, Bacteroidetes were more abundant in the dry season (37.0%) and represented mainly by Cytophagia class (31.0%) and *Pseudarcicella* OTU (30.0%). Among Firmicutes, Bacilli

prevailed during the dry season (6.4%) and *Enterococcus* was the most predominant OTU (4.2%) (Fig. 2A, B).

In contrast, Firmicutes prevailed in treated water samples obtained in both rainy and dry seasons. Relative abundance of this phylum was 60.4% for PTW and 79.9% for TW and 99.0% for PTW and 99.2% for TW in the rainy and dry seasons, respectively. Relative abundances detected for other phyla (presented for PTW rainy and dry seasons and TW rainy and dry seasons, respectively) are as follows: Proteobacteria (8.6% and 9.8%, 0.5% and 0.5%), Actinobacteria (12.0% and 0.5%, 0.1% and 0.1%), Deinococcus-Thermus (3.4% and 6.8%, 0.0% and 0.0%), Cyanobacteria (6.4% and 0.4%, 0.09% and 0.05%), Planctomycetes (5.1% and 0.02%, 0.02% and 0%), and Bacteroidetes (2.5% and 1.4%, 0.09% and 0.05%) (Fig. 1).

Bacilli was the most dominant class in PTW and TW, with relative abundances of 53.0% and 79.0% in the rainy season and 98.0% and 98.2% in the dry season, respectively. *Geobacillus* [33.5% (PTW) and 66.3%(TW)] and unclassified Bacillaceae [9.0% (PTW) and 12.0%(TW)] were the most abundant OTUs in the rainy season, while *Enterococcus*, unclassified Bacillales, and *Paenibacillus* prevailed in the dry season constituting 62.0%, 26.9%, and 3.2% of PTW and 57.9%, 29.0%, and 5.0% of TW, respectively. Within the phylum Proteobacteria, Alphaproteobacteria was the most abundant class particularly in the rainy season (4.0% for PTW and 5.0% for TW). Within this class *Methylobacterium* should be highlighted (1.0% for PTW and 2.5% for TW). A high proportion of *Mycobacterium* OTU (12.0%) included in the phylum Actinobacteria was observed for PTW but not for TW (0.5%). A single Deinococci OTU, assigned as *Thermus* was detected in PTW and TW in the rainy season (3.4% and 6.8%, respectively) (Fig. 2A, B).

Shared and unique OTUs detected in the three sampled points of DWTS are illustrated in a Venn diagram (Fig. 3). RW had the larger number of exclusive OTUs (2,396 OTUs), followed by PTW (839 OTUs), and TW (295 OTUs). Shared bacterial components were observed as follows: 114 OTUs between RW and PTW, 180 OTUs between PTW and TW, and 165 OTUs between TW and RW. Common bacteria found in all three points comprised

138 OTUs, which belong mainly to *Enterococcus* (OTU 001), *Geobacillus* (OTU 003), Bacillales_unclassified (OTU 002), Bacillaceae_unclassified (OTU 010), and *Paenibacillus* (OTU 015) (phylum Firmicutes); *Comamonadaceae*_unclassified (OTU 005), *Undibacterium* (OTU 008), and *Neisseriaceae*_unclassified (OTU 007) (phylum Proteobacteria, class Betaproteobacteria); *Methylobacterium* (OTU 038) and *Blastomonas* (OTU 025) (phylum Proteobacteria, class Alphaproteobacteria); *Acinetobacter* (OTU 006) and *Pseudomonas* (OTU 026) (phylum Proteobacteria, class Gammaproteobacteria); *Thermus* (OTU 018) (phylum Deinococcus-Thermus); and *Mycobacterium* (OTU 047) (phylum Actinobacteria) (Fig. 3).

3.4 Bacterial community diversity and bacterial quantification changes in DWTS

The alfa diversity, beta diversity, and bacterial quantification were used to confirm treatment effect on the bacterial community. Profile of OTUs and Shannon and Chao1 indices were used to determine community alfa diversity. Higher values were observed for RW samples when compared to treated water samples. Figure 4 shows a progressive decrease in all indices throughout DWTS, except in dry season when similar results were observed for PTW and TW. PCoA using the unweighted UniFrac distance revealed significant changes in the bacterial community when untreated and treated water samples where compared. In contrast, communities of PTW and TW were very similar, particularly in the dry season (Fig. 5).

Additionally, bacteria count decreased from 1×10^6 and 6×10^6 16S rRNA gene copies/µL in RW samples obtained in the rainy and dry seasons, respectively to 5×10^3 and 3×10^3 copies/µL in PTW samples. After distribution of the drinking water, bacterial community quantification in TW exhibited 1×10^4 copies/µL in the rainy season and 2×10^3 copies/µL in the dry season (Table 2).

3.5 Characterization of potential pathogenic bacterial communities

Several genera including potential pathogens were detected in water samples by sequencing analysis (Fig. 6), notably, *Acinetobacter*, *Pseudomonas*, *Escherichia-Shigella*, *Mycobacterium*, *Enterococcus*, *Arcobacter*, *Legionella*, and *Aeromonas*. A BLASTn search in PATRIC 16S rRNA gene database showed highly significant alignments of these OTUs with *A. baumanni* (ID1310571.3.rna.49), *P. aeruginosa* (ID 1365011.3.rna.50), *Escherichia coli* (ID 585055.8.rna.1), *Mycobacterium intracellulare* (ID 1299331.3.rna.14)*, Enterococcus faecalis* (ID 565646.5.rna.36), *Methylobacterium* sp*.* (ID 1736245.3.rna.26), *Legionella* sp. (ID 1034944.4.rna.6), *Arcobacter* sp. (ID 1355371.3.rna.8), and *Aeromonas hydrophila* (ID 596318.3.rna.7). In comparison with the other OTUs, the relative abundance of *Methylobacterium* sp., *A. baumanni, E. faecalis*, *E. coli,* and *M. intracellulare* increased after treatment (PTW) and *Methylobacterium* sp. and *A. baumannii* also in TW at the rainy season and *E. faecalis* in TW at the dry season (Fig. 6).

4 Discussion

The detection of total coliforms, *E. coli*, and heterotrophic bacteria count in culture medium is usually employed to assess the overall bacterial quality of drinking water systems (WHO, 2011; Douterelo et al., 2014). However, this view hampers the understanding of the true composition and dynamics of bacterial communities and diversity in DWTS (Doutorelo et al., 2016). Several recent studies have provided a comprehensive view of bacterial diversity from RW source until point of use of DWTS based on new generation sequencing tools (Chao et al., 2013, Bai et al., 2015; El-chakhtoura et al., 2015; Stanish et al., 2016), contributing to a more comprehensive microbiological knowledge of drinking water treatment. Nevertheless, we are not aware of such kind of study conducted in a DWTS that uses a different type of RW as the present investigation. Also, we could not find in the available literature any reports targeting bacterial communities exploring through metagenomics approach carried out in Brazil.

In the present study, Proteobacteria, particularly Betaproteobacteria were dominant in RW samples (Fig 1, 2A). Previously reported data (Pinto et al., 2012; Holinger et al., 2014; Proctor and Hammes, 2015; Vaz-Moreira et al., 2017) also demonstrated that Betaproteobacteria is more abundant in RW, while Actinobacteria, Firmicutes, Verrucomicrobia, Nitrospirae, and Bacteroidetes are less represented (Karwautzand Lueders, 2014; El-chakhtoura et al. 2015). Not surprisingly the more abundant OTU in RW in both seasons was *Comamonadaceae*_unclassified (Fig. 2B), part of a ubiquitous family described as a dominant bacterial group in freshwater environments (Newton et al., 2011). Differently, in treated water samples (PTW and TW) Firmicutes phylum, particularly Bacillus class prevailed. Data generated showed that the *Geobacillus* (OTU 003) and unclassified *Bacillaceae* (OTU 010) (rainy season) and *Enterococcus* (OTU 001), unclassified Bacillales (OTU 002), and *Paenibacillus* (OTU 015) (dry season) were present in RW and more than persist, their relative abundance increased after water treatment (Fig. 1, 2A, B, 3). Similarly, Sun et al. (2014) and Li et al. (2017) also reported an increased abundance of Firmicutes in chlorinated water distribution systems when compared to untreated water. Stanish et al. (2016) observed some genera, e.g. *Bacillus* persisting after conventional drinking water treatment and reported the shift of bacterial community from Alphaproteobacteria and Betaproteobacteria to Firmicutes and Gammaproteobacteria.

Despite that, factors that regulate the abundance of Firmicutes in drinking water are rarely addressed (Sun et al., 2014, Wu et al., 2015) and there is little information on the impact of disinfectants such as chlorine on this phylum or its possible resistance to water treatment processes (Sun et al., 2014, Mi et al., 2015). Firmicutes has generally been found in a smaller proportion in drinking water supply systems (Kormas et al., 2010; Pinto et al., 2012, Vaz-Moreira et al., 2013; Ng et al., 2015). Taxa Bacillales, *Bacillaceae*, *Geobacillus*, and *Paenabacillus* produce highly resistant endospores, which can provide a selective advantage in treatment systems and in relatively high concentrations of chlorine. This hypothesis is in line with observations made by Ridgway and Olson (1982) that spore-forming taxa such as *Bacillus* are much more resistant to chlorine than those that do not

328 sporulate. However, some results indicate that even non-spore-forming Gram positive
329 bacteria may express higher chlorine resistance (Mir et al., 1997).
330 The OTU detected as *Enterococcus* (Otu001) was identified by PATRIC database as
331 *Enterococcus faecalis* (ID 565646.5.rna.36). Studies on the resistance of *E. faecalis* to
332 disinfection, such as chlorination employed in wastewater treatment shows that the
333 bacterium survives to the process although a density decay is observed (Tree et al., 2003;
334 Rosenberg Goldstein et al., 2014). It is also important to highlight that some recent studies
335 have shown that water disinfection imposes a stress on the microbial community that may
336 be associated with an increase in the prevalence of antimicrobial resistant bacteria (Shi et
337 al., 2013; Bai et al., 2015). *E. faecalis* stands out as an opportunistic pathogen that
338 expresses intrinsic and acquired resistance to several antimicrobial drugs, what may help
339 explaining its persistence throughout DWTS (Byappanahalli et al., 2012).
340 Betaproteobacteria was highly vulnerable to water treatment as demonstrated by a
341 dramatically decreased relative abundance in PTW (Fig. 2A). The result is in accordance
342 with previous reports that described this bacterial class as more susceptible to DWTS
343 (Williams et al., 2004; Hwang et al., 2012). Interestingly, the abundance of the other
344 dominant classes in treated water samples, such as Actinobacteria, Clostridia, and
345 Melainabacteria increased immediately after treatment (PTW) in the rainy season but
346 decreased subsequently (TW), showing the instability of these groups along the distribution
347 system (Fig. 2A). *Mycobacterium*, the most abundant Actinobacteria OTU (Fig. 2B) is
348 consistently reported to be resistant to chlorine (Falkinham, 2015).
349 In contrast, the abundance of Alphaproteobacteria and Deinococci classes decreased in a
350 smaller proportion after treatment (PTW) in the rainy season, and increased along the
351 distribution, with greater abundance in TW (Fig. 2A), demonstrating the stability of these
352 groups in water even those containing residual chlorine. Li et al. (2017) also reported
353 increased of Deinococci relative abundance after drinking water treatment. This class
354 includes coccoid-shaped bacteria highly resistant to extreme environmental conditions
355 (Griffiths and Gupta, 2007), such as *Thermus*, the representative OTU of this group found

356 in this study (Fig. 2B). Alphaproteobacteria is traditionally reported as a persistent taxon
357 after water treatment, because it may tolerate high chlorine concentrations as well as low
358 levels of nutrients. These abilities confer an advantage towards survival in the tap water
359 environment (Chao et al., 2013; Chao et al., 2015; Vaz-Moreira et al., 2017).
360 *Methylobacterium*, the OTU more representative of this class (Fig. 2B) presents a high
361 resistance to chlorination (Kovaleva et al., 2014).
362 The treatment exerted effects not only on the structure of the water bacterial community,
363 but it also promoted disturbances that resulted in decreased diversity (Fig. 5). PCoA using
364 the unweighted UniFrac distance confirmed this result and revealed significant changes
365 when the community of RW was compared to those detected in treated water samples
366 (PTW and TW; Fig. 6). Other studies also found profound changes in the biological profiles
367 of RW (Chao et al., 2013; Zhang et al., 2017). In addition, the quantification of the 16S rDNA
368 shows the reduction of bacterial count in post-treated water samples, as expected (Table
369 2). These results confirm that the treatment process in DWTS Itacolomi influences the
370 microbioma and similar patterns in microbial composition between the treated water
371 samples (PTW and TW) observed, particularly in dry season, indicates that the distribution
372 system proved to be relatively stable until its end point.
373 Importantly, reads of *Escherichia-Shigella* and *Enterococcus* were detected in drinking
374 water distribution systems and classified by PATRIC database as *E. faecalis* and *E. coli*. In
375 fact, *E. coli* and *E. faecalis* were detected in all water samples, mainly in the rainy and dry
376 seasons, respectively (Fig. 6). This result evidentiates an undesirable contamination of
377 water systems possibly due to fecal contamination originated from RW (WHO, 2011).
378 However, recent studies have shown that *E. coli* and *Enterococcus* can also be found in
379 natural environments not exposed to anthropogenic influences, including freshwater (Ran
380 et al., 2013; Weigand et al., 2014; Di Sante et al., 2018).
381 Genera including pathogenic species, such as *Mycobacterium*, *Acinetobacter*,
382 *Pseudomonas*, *Methylobacterium*, *Legionella*, *Arcobacter*, and *Aeromonas* were also
383 detected in treated water samples (PTW and TW) and classified by PATRIC database as

M. intracellulare, *A. baumannii*, *P. aeruginosa*, *Methylobacterium* sp., *Legionella* sp., *Arcobacter* sp., and *A. hydrophila*. In this study, *M. intracellulare*, *A. baumannii, E. faecalis*, *E. coli*, and *Methylobacterium* sp. showed particularly relevance since they increased their proportion in treated water samples (Fig. 6). This finding suggests that they could resist to drinking water treatment processes and persist even in the presence of chlorine in the distribution system. Indeed, these microorganisms are very complex and frequently described as resistant to disinfectants (Ashbolt, 2015). The occurrence of these pathogenic bacteria in drinking water may increase the risk for water-related diseases. This is particularly relevant if we take into account the environment where TW samples were collected, the main Ouro Preto hospital, considering the kind of individuals threatened by unsafe water (Ashbolt, 2015). These four taxa are not rare associated to several infectious diseases. For example, *E. faecalis* cause opportunistic infections, mainly urinary tract infection (Guzman Pietro et al., 2016), *M. intracellulare* and *A. baumannii* can lead to respiratory diseases (Billinger et al., 2009; Davis et al., 2014), and *Methylobacterium* sp. reportedly cause bacteremia (Kovaleva et al., 2014). Although the presence of reads corresponding to these pathogenic species does not confirm their viability, their presence clearly raises concern for the public health.

5 Conclusions

This is the first DWTS study conducted in Brazil using 16S rRNA gene deep sequencing. Furthermore, this is one of the very few studies that demonstrate the possible persistence of Firmicutes in DWTS. Interestingly, in this investigation Gram positive bacteria included in the phylum Firmicutes, class Bacilli demonstrated versatility by persisting and dominating post-treatment samples of water derived from a DWTS in both rainy and dry seasons. The main OTUs representative of these groups were the spore formers *Geobacillus*, unclassified Bacillales, unclassified *Bacillaceae*, *Paenibacillus*, and *E. faecalis* that expresses enormous capacity to acquire antimicrobial resistance markers. In addition, this study confirms the

effects of water treatment on bacterial community structure and diversity and the persistence of potential pathogens in DWTS, which is alarming because it can endanger the population that consumes such waters, mainly immunocompromised individuals as is the case of this investigation.

Authors' contributions

AGGO, LMF, PPM, MCS and AMAM designed the study. AGGO, JFGF and CPS performed sample collection. AGGO, JFGF and CPS performed samples processing and physicochemical analysis. AGGO conducted DNA extraction; AGGO and MFD library preparation. AGGO and MFD carried out the bioinformatics and statistical analysis. PPM, LMF, AMAM, and AGGO wrote the manuscript. All authors read and approved the final version of the manuscript. All authors declare that they have no competing interests.

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Table 1. Physicochemical characteristics of raw water (RW), post-treatment water (PTW), and tap water (TW) of a drinking water treatment system.

Sample	Temperature (°C)	pH	Turbidity (NTU)	TS (mg/L)	TDS (mg/L)	DO (mg/L)	Free chlorine (mg/L)	Nitrate (mg/L as NO_3^-)	Nitrite (mg/L as NO_2^-)	Phosphate (mg/L as PO_4^{3-})	BOD (mg/L)	COD (mg/L)
RWrainy	19.5	7.7	1.32	0.05	33.3	8.0	0	2.0	0.005	0.15	0.04	75
PTWrainy	24.5	8.6	0.04	0.01	38.0	7.5	2.5	2.6	0.005	0.19	0.09	60
TWrainy	24.8	7.7	0.04	0.001	13.6	7.7	0.4	2.2	0.003	0.16	0.1	115
RWdry	18.5	5.5	1.00	0.02	31.7	8.6	0	1.9	0.004	0.18	0.9	1.35
PTWdry	21.5	5.9	0.04	0.01	17.9	8.8	1.2	2.2	0.003	0.18	0.05	0.08
TWdry	22.5	6.9	0.04	0.01	92.4	7.8	1.0	2.4	0.002	0.20	0.03	0.05

TS: total solids; TDS: total dissolved solids; DO: dissolved oxygen; BOD: biochemical oxygen demand; COD: chemical oxygen demand.

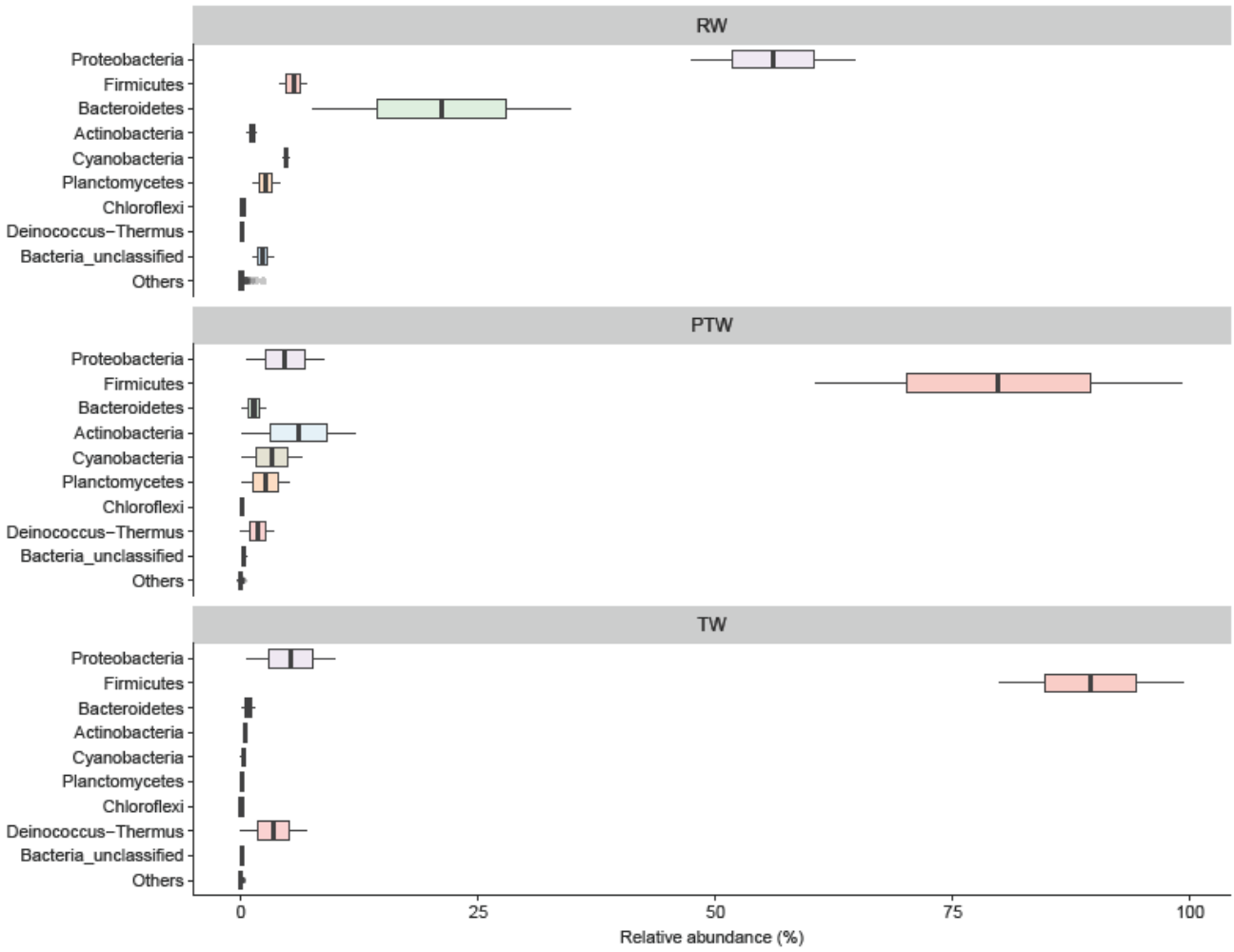

Figure1. Relative abundance of nine most abundant phyla of the DWTS in rainy and dry seasons. RW: raw water; PTW: post-treatment water; TW: tap water.

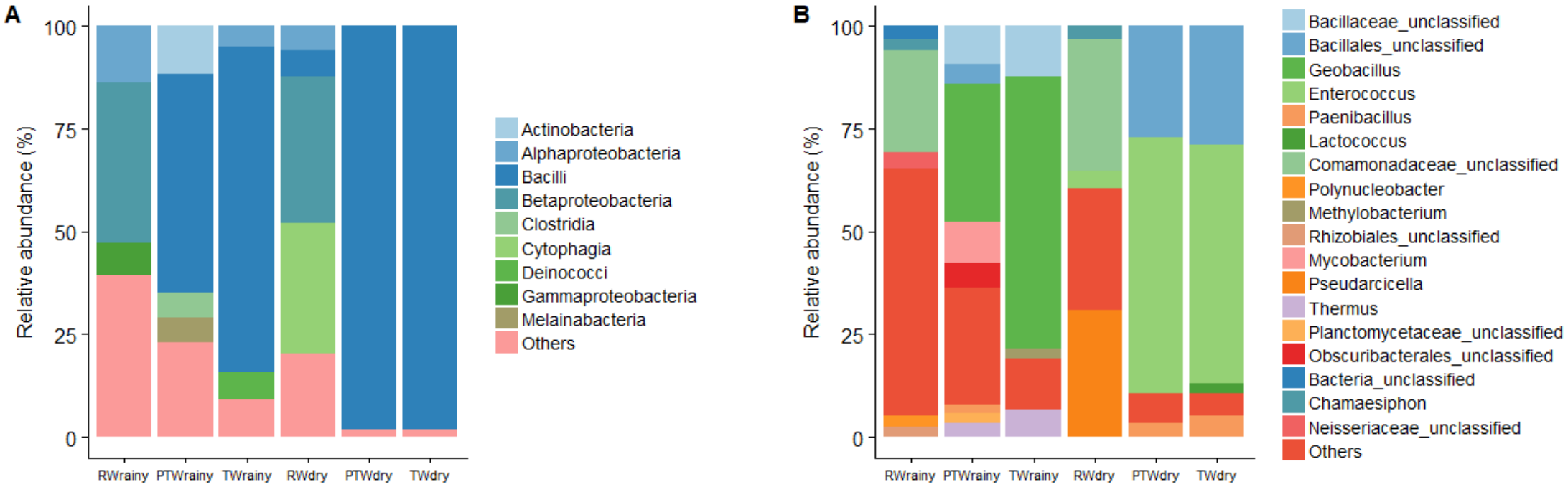

Figure 2. Relative abundance of A) nine abundant classes and B) eighteen most abundant operational taxonomic units (OTUs) at the lowest taxonomic level possible of a drinking water treatment system in rainy and dry seasons.RW: raw water; PTW: post-treatment water; TW: tap water

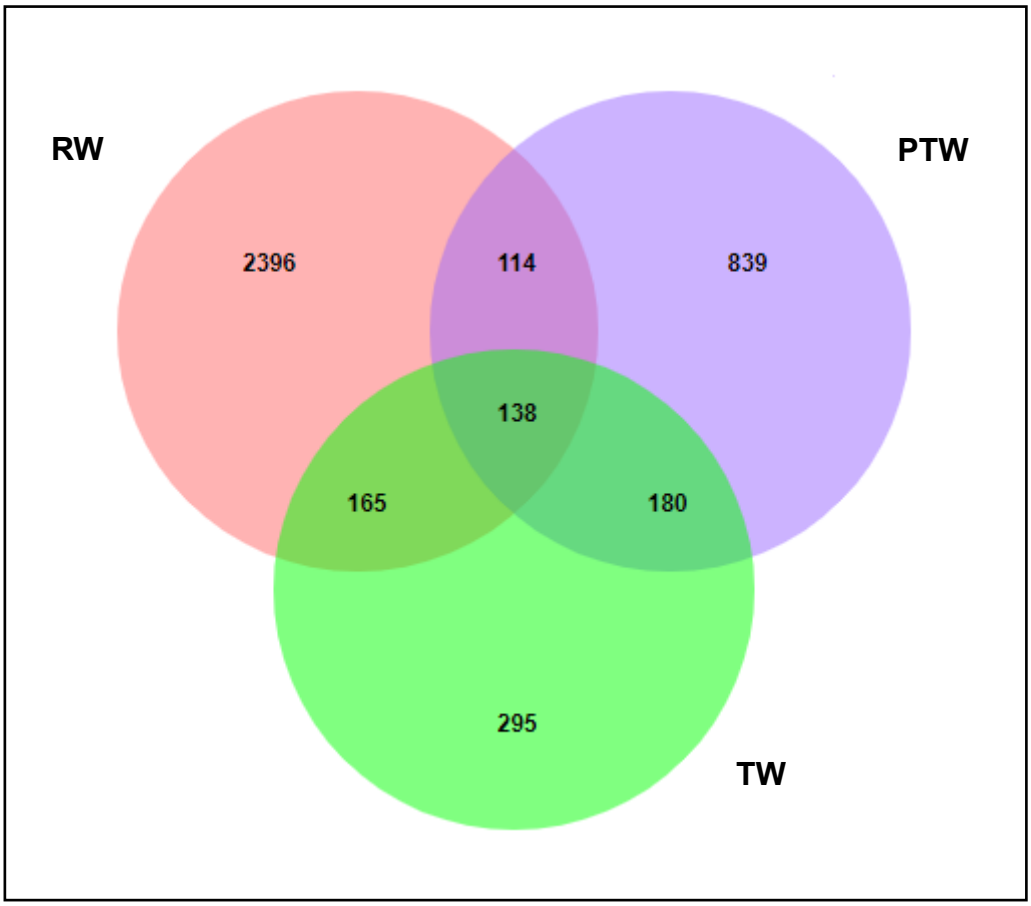

Figure 3. Venn diagram showing the number of shared and unique operational taxonomic units (OTUs) in the three drinking water treatment system sampled points. RW: raw water; PTW: post-treatment water; TW: tap water.

Table 2. Bacterial quantification in raw and treated water using 16S rRNA gene qPCR.

Sample	rRNA 16S gene (copies/µL)
RWrainy	1×10^6
PTWrainy	5×10^3
TWrainy	1×10^4
RWdry	6×10^6
PTWdry	3×10^3
TWdry	2×10^3

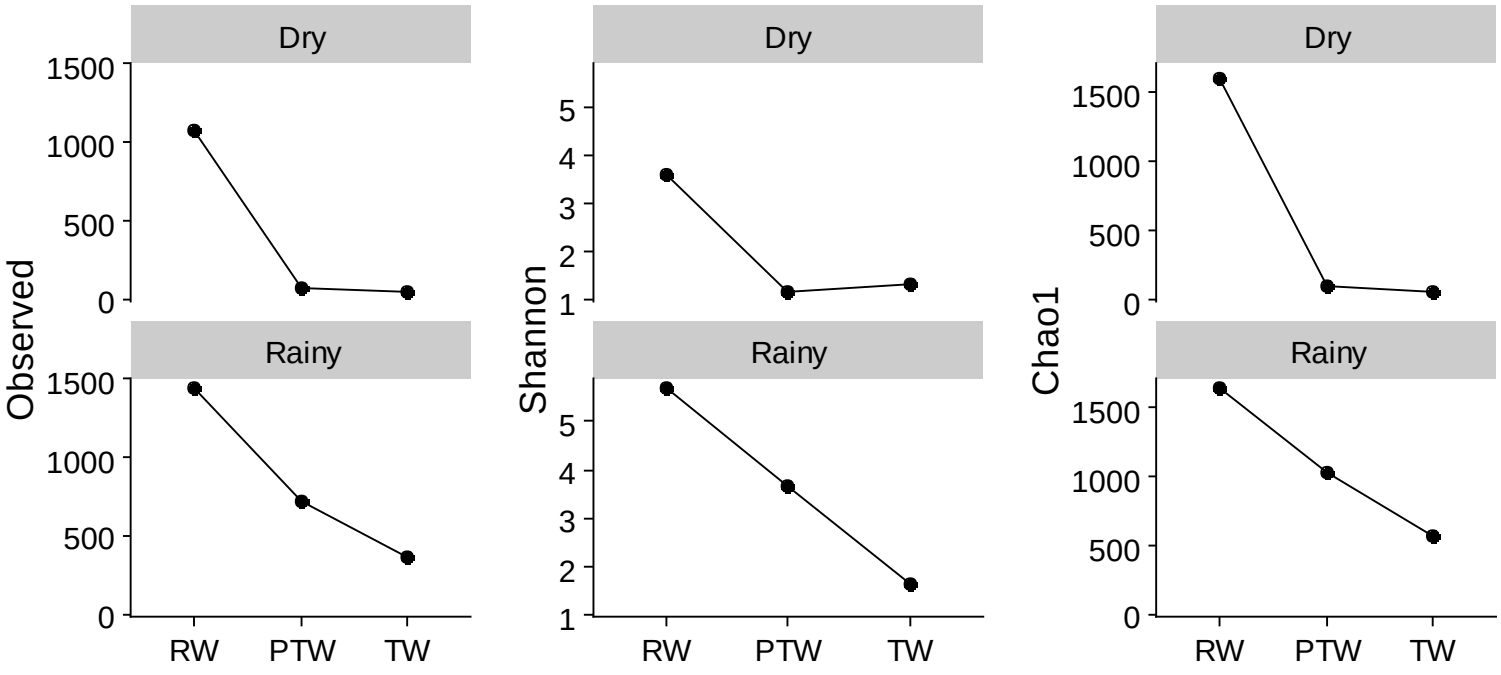

Figure 4. Alfa-diversity indices. Number of OTUs, Chao1 index, and Shannon index for bacterial communities in a distribution water treatment system. RW: raw water; PTW: post-treatment water; TW: tap water.

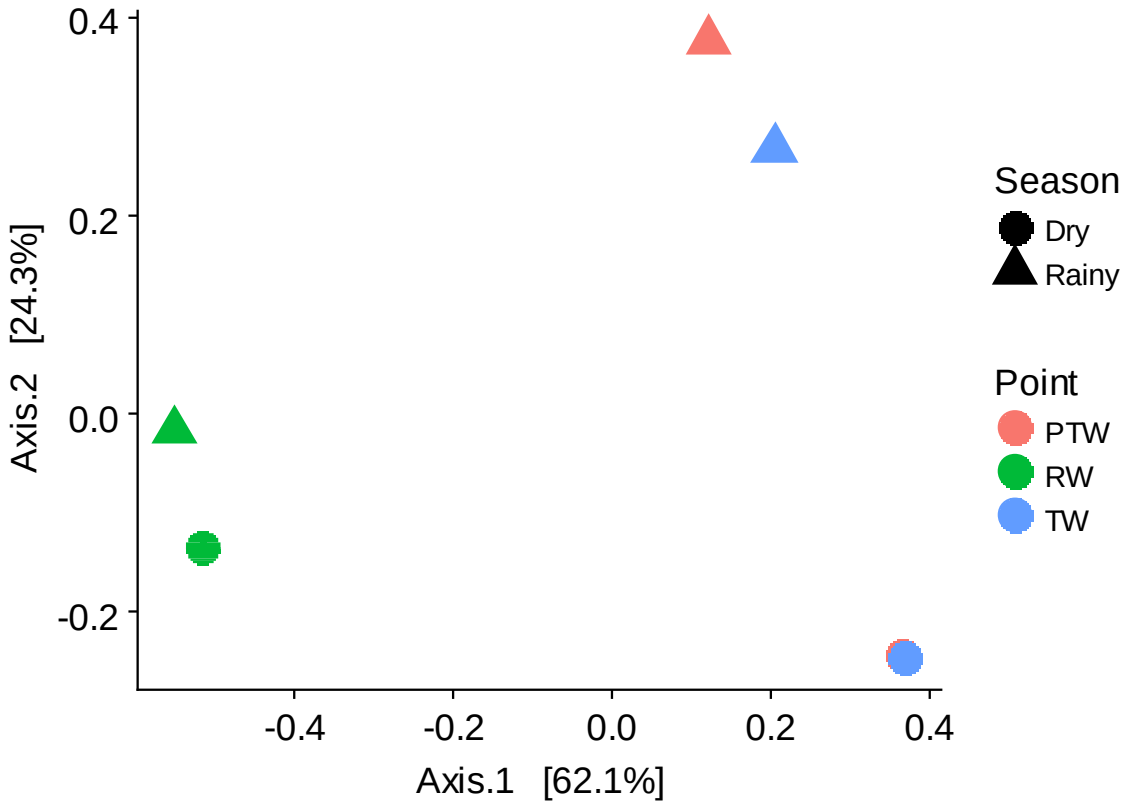

Figure 5. Beta-diversity of the microbial community in a drinking water treatment system. Beta-diversity was represented with unweighted UniFrac unweighted distances using principal coordinates analysis (PCoA), illustrating the variability of water samples collected in rainy and dry seasons. Data points are colored according to sampling positions. RW: raw water; PTW: post-treatment water; TW: tap water.

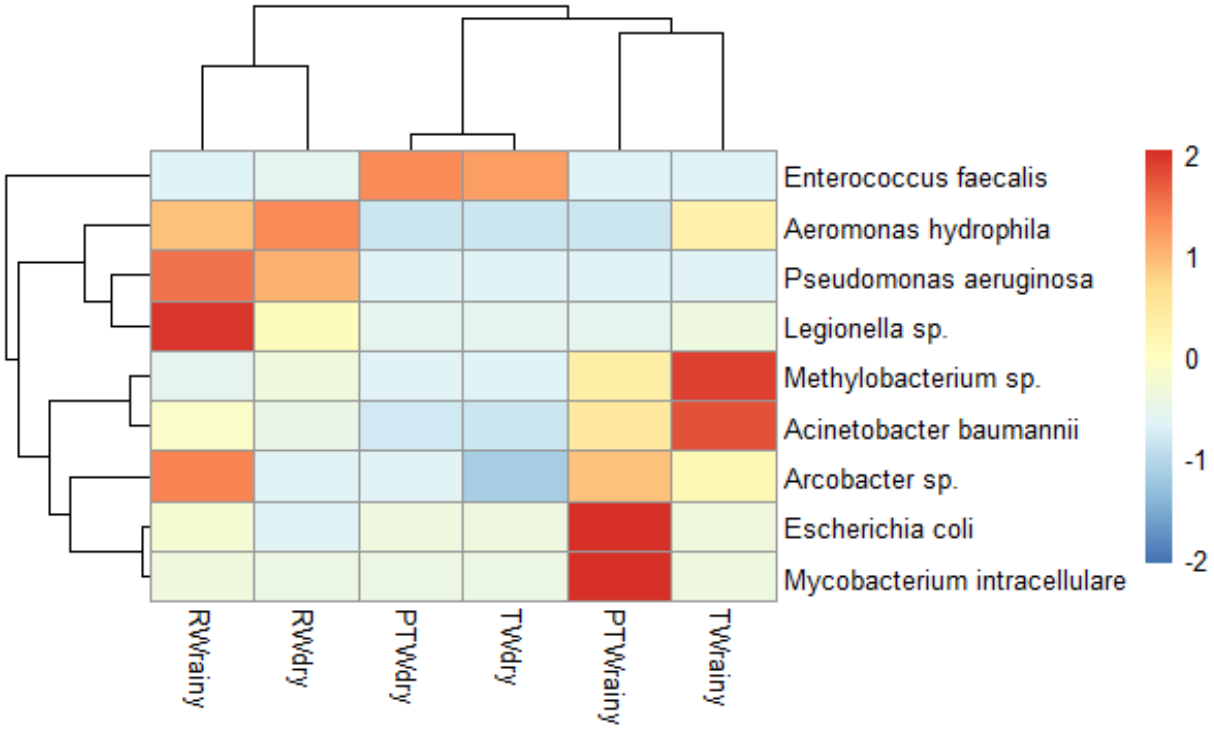

Figure 6. Heatmap of relative abundance of potential pathogenic OTUs detected in a drinking water treatment system in rainy and dry seasons. RW: raw water; PTW: post-treatment water; TW: tap water. Scale bar shows the variation range of the normalized abundance of the OTU.

4.2 ARTIGO 2

ARTIGO A SER SUBMETIDO AO PERIÓDICO *SCIENCE OF THE TOTAL ENVIRONMENT*

Procaryotic community composition and diversity in freshwater sources used as drinking water in a Brazilian tourist city

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Keywords: freshwater, procaryotic communities, water metagenomics.

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Abstract

Water sources harbor a high procariotic diversity that is directly related to public health safety. In this study, the procaryotic community taxonomic composition and diversity of freshwater was investigated by 16S rRNA gene deep sequencing. Surface water and groundwater employed as alternative water sources (no treatment) or used to feed the official drinking water treatment system in Ouro Preto, an important touristic city in southeastern Brazil were evaluated. Procaryotic diversity and taxonomic composition of surface water sources showed no seasonality. In contrast, the diversity and structure of Bacteria and Archaea communities of groundwater sources were significantly changed in the rainy season. In regard to taxonomic composition Proteobacteria phyla, Betaproteobacteria class were the dominant taxa found in all samples except for groundwater in the dry season that exhibited a predominance of Firmicutes phyla, Bacilli class. The search for correlation between water quality parameters and procaryotic community structure showed a strong association between Bacteroidia, Clostridia, Gammaproteobacteria, and Flavobacteriia classes and organic pollution. Bacilli, South African Gold Mine, Nitrospira, and Acidobacteria were negatively associated with pH and dissolved oxygen and, except for Bacilli, they were positively related to arsenic and nitrate. Alphaproteobacteria, Betaproteobacteria, and Cytophagia were positively associated with surface water sources that present adequate physicochemical parameters, such as oxidation reduction potential, pH, and dissolved oxygen. *Bacillus*, *Enterococcus*, *Ralstonia*, and *Acinetobacter* among other OTUs were positively correlated with heavy metals. The detection of several well-recognized potential pathogens, including some classically water vehiculated organisms alerts for the risk imposed by freshwater mainly those that are used as alternative drinking water sources (not submitted to any treatment), specially for more susceptible individuals such as immunocompromised hosts.

1. Introduction

Surface water and groundwater are important freshwater ecosystems that harbor a complex microbial diversity and are often used as drinking water sources, what directly links microbiological water quality to public health. In addition to natural occurring organisms, anthropogenic environmental disturbances lead to changes in water microbiological profile what may threat people wellness management. This problem is aggravated by disparities in population access to safe water supplies and basic health care services in developing countries like Brazil (Haseena et al., 2017).

Traditionally, fecal indicators such as total coliforms, *Escherichia coli*, and *Enterococcus* have been used as the "gold standard" in the assessment of microbial safety of drinking water (WHO, 2011; Boehm and Sassoubre, 2014). However, the approach do not allow the assessment of the true potential risk of the water because data generated do not necessarily correlate with the presence of other important pathogens, such as *Legionella*, *Mycobacterium*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (Figueras and Borrego, 2010; Wang et al., 2012). Taking this into account several authors have been using high-throughput sequencing technologies to investigate bacterial community diversity in drinking water sources, providing new insights into the microbial ecology, revealing the presence of a vast diversity of microorganisms previously undetected and consequently expanding the knowledge about microbial communities of drinking water (Lee et al., 2016; Liu et al., 2016; Uyaguari-Diaz et al., 2016).

Ouro Preto, a city in the state of Minas Gerais was the focal point of the gold rush and Brazil's golden age in the 18th century and the first Brazilian city to receive the title of Intangible Cultural Heritage of Humanity by UNESCO, being part of the international tourism route ((https://whc.unesco.org/en/resources/). To date, the city is still an important mining region. However, the activity causes environmental degradation, affecting the surface water and groundwater that are used for public supply (Pimentel et al., 2003; da Costa et al., 2015). In

addition, the city faces several problems, such as the lack of systematic and comprehensive monitoring of the quality of water provided to the population in order to ensure Brazilian drinking water standards (MS, 2011). Although there are official public drinking water treatment systems (DWTS) in Ouro Preto, alternative sources (untreated water) are still frequently used by the population because official supplier does not generate enough treated water for satisfying population needs. Additionally, using untreated water is a well-established habit of the local population built upon the belief that water sources of the region are safe. These include surface water and groundwater, such as tubular wells and abandoned gold mines, for domestic, public or institutional supply (Fonseca and Prado, 2006). Therefore, the microbial diversity of these waters should be analyzed to provide a better understanding of their public health safety.

Although there are many studies concerning water quality in Brazil (Orsi et al., 2007, Gomes et al., 2017, Alves et al., 2018, Ribeiro et al., 2018), few of them have been undertaken in drinking water sources (Nogueira et al., 2002; Rocha et al., 2006; de Siqueira et al., 2010; Alves et al, 2014). In addition, most studies used fecal indicators to access water quality, while other opportunistic bacteria are rarely investigated (Silva et al, 2008; Falcone-Dias et al., 2015) and mainly there are no studies that focused on a broader microbial ecological analysis that employed high-throughput sequencing technologies in freshwater sources used as drinking water in Brazil.

In this study, we explored the procaryotic abundance and diversity of surface water and groundwater sources used by official supply systems as well as directly by the population as alternative water sources in Ouro Preto, an important tourist city in southeastern Brazil, using 16S rRNA gene deep sequencing. Results generated were confronted with several water quality parameters on a seasonal basis and potential pathogens were also investigated.

2. Materials and methods

2.1 Water sampling locations

Water sources selected for the study constitute five of the more important freshwater sources used for public supply in Ouro Preto, Minas Gerais, Brazil (20.23°S, 43.30°W), including official (Itacolomi, surface water and Mina Rainha, groundwater) and alternative sources [Buraco Quente and Novo Horizonte, surface water and Universidade Federal de Ouro Preto (UFOP), groundwater]. The selected water sources were GPS located using the Google Earth software (Figure 1 and Table 1).

2.2 Sample processing

Water samples were collected at the point of distribution for consumption, for alternative water, or at the point of distribution before treatment, for officially distributed water. Sampling was repeated two times, in rainy (February 2016) and dry (August 2016) seasons. Rainfall rates for each period (month) were 166 mm and 17 mm, respectively (www.climatempo.com.br/ouropreto).

From each studied site, water samples were collected in two sterile bottles, one for microbiological and the other for physicochemical analyses. For microbiological evaluation, 2 to 10 L of water were filtered through a 0.22 mm nitrocellulose membrane (Millipore, USA) and stored at -80 °C until DNA extraction.

2.3 Physicochemical analysis

Temperature, pH, conductivity, dissolved oxygen (DO), and oxidation reduction potential (ORP) were measured in all samples using a HQ40D Portable Multi Meter (Hach, USA) and turbidity was determined using the 21100Q Portable Turbidimeter (Hach, USA), affered in situ. Total solids (TS), nitrate, nitrite, ammonia, phosphate, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) were analyzed as recommended by the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2012). Heavy metals -

manganese (Mn), Iron (Fe), lead (Pb), chromium (Cr), cadmium (Cd), nickel (Ni), and arsenic (As) - were determined using the atomic absorption spectrometer, OPTIMA 2000 DV ICP Optical Emission Spectrometer (Perkin Elmer, EUA).

2.4 DNA extraction and Illumina sequencing

Total DNA was extracted with the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, USA), according to the manufacturer's instructions, and quantified with Qubit® fluorometer (Thermo-Fisher Scientific, USA). 16S rRNA gene amplicon analysis was carried out using paired-end libraries with the universal primer pair targeting the V4 hypervariable regions of both Bacteria and Archaea domains (515F: 5'-GTGYCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACNVGGGTWTCTAAT-3') (Caporaso et al., 2012; Klindworth et al., 2013). The library construction was performed following the protocol provided by Illumina for 16S rRNA gene library construction (16S Metagenomic Sequencing Library Preparation, 2013). Sequencing was performed at a Miseq platform, with the MiSeq Reagent Kit v2 (500 cycles).

2.5 Sequence analysis

The paired reads were trimmed to remove bases with Phred score lower than five at the 5′ and 3′ extremities. These procedures also trimmed sequences with an average quality <15 in a sliding window of 4 bases. The software Trimmomatic (Bolger et al., 2014) performed this quality filtering. Paired reads were merged using the FLASh tool (Magoc and Salzberg, 2011), requiring a minimum overlap of 20 nucleotides. Redundancy and unique entries were removed using the dereplication step from Vsearch (Rognes et al., 2016). The reads were mapped to the dereplicated sequences to create clusters with a 97% identity threshold using the same tool to create the OTUs. Taxonomical assignment to the OTUs was performed by the assign_taxonomy script from Qiime (Caporaso et al., 2010) and Silva database, version 123 (Quast et al., 2013). The affiliation of potential pathogens at the species level was analyzed

through a BLASTn search in PathoSystems Resource Integration Center (PATRIC) 16S rRNA gene database with 100% of sequence coverage and identity.

2.6 Statistical analysis

Alpha and beta-diversity analyses were conducted with the R packages Phyloseq and Vegan (Oksanen et al., 2008; McMurdie and Holmes, 2013). After rarefying the number of reads, observed OTUs, Chao1 estimator, and Shannon indexes were calculated with the estimate_richness function in Phyloseq. Significant differences in alpha-diversity measures and physicochemical parameters were tested with generalized linear models (GLM) by using R platform. The residual analysis of the models was conducted with RT4Bio package (Reis Jr. et al., 2015) in order to verify the best error distribution. Unweighted UniFrac distances (Lozupone et al., 2010) were determined and ordinated by principal coordinate analysis (PCoA). DESeq2 package (Love et al., 2014) was used to test for significant differences in phylum and OTU abundance between freshwater sources. Canonical correspondence analysis (CCA) was selected to compare class-environment correlations using Vegan, and ANOVA permutation (number of permutation = 999) was used to test the model. Person's correlation analysis was used to establish correlations between OTU and heavy metals parameters using Vegan.

3. Results

3.1 Physicochemical properties of water sources

The water quality parameters are shown in S1. Most of the parameters analyzed did not exceed the limits established by the Brazilian legislation for drinking water (MS, 2011).

The differences between water types and water sources were assessed by using generalized linear models (GLM). The parameters TS (total solids), ammonia, nitrite, conductivity, ORP,

and phosphate did not show significant differences among the samples ($p > 0.05$). Buraco Quente exhibited some indexes above the limit imposed by Brazilian legislation. Some parameters such as temperature, turbidity, BOD, and COD showed higher values than those detected in the other water sources ($p < 0.05$). Groundwater sources showed lower pH and DO and higher levels of As when compared to surface water sources ($p < 0.05$). Pb, Cr, and Cd were not detected in any water sample. In contrast, Mn, Fe, As, and Ni showed elevated levels in several water sources. The season influenced significantly only COD, that increased in the rainy season ($p < 0.05$).

3.2 Diversity of procaryotic community of water sources

The number of generated and processed reads is shown in S2. Alpha-diversity index, based on observed OTUs, Chao1 estimator, and Shannon diversity index were calculated and significant differences between water types were assessed by using GLM. Alpha-diversity metrics are shown in Figure 2. Regarding the comparison between diversity of different water types, the measurements varied significantly between surface water and groundwater only in the dry season, in which richness and diversity of surface water were higher than that of groundwater ($p < 0.05$). In regard to the influence of the climatic season, the richness and diversity of groundwater were higher in the rainy than in the dry season ($p < 0.05$), whereas no seasonality was observed for surface water ($p > 0.05$) (Fig. 2).

PCoA ordination of unweighted UniFrac distances revealed that the procaryotic communities of surface water sources were more similar regardless of the season than that of groundwater sources. Groundwater sources showed similar results in the dry season, but not in the rainy season, revealing that seasonal variations may affect groundwater microbioma (Fig. 3).

3.3 Taxonomic structure of procaryotic communities in water sources

Procaryotic communities of surface water sources were dominantly composed of the phylum Proteobacteria (72.0% ± 18.2%), mainly class Betaproteobacteria (50.0% ± 25.0%), followed by Gammaproteobacteria (11.0% ± 11.0%) and Alphaproteobacteria (5.0% ± 4.0%). The phylum Bacteroidetes (11.6% ± 11.3 %) was also abundant, mainly classes Bacteroidia (3.0% ± 3.0%), Flavobacteriia (4.0% ± 5.0%), and Cytophagia (6.0% ± 11.0%), followed by phylum Firmicutes (6.4% ± 5.8%), mainly represented by classes Bacilli (2.0% ± 2.0%) and Clostridia (3.0% ± 4.0%) (Fig. 4, 5). Cyanobacteria (2.0% ± 2.0%), followed by Actinobacteria, Planctomycetes, and Verrucomicrobia (approximately 1% of relative abundance each) were less abundant in surface water (Fig. 4).

In contrast, groundwater sources were dominantly composed of Proteobacteria (50.0% ± 13.2 %), mainly represented by the class Betaproteobacteria (35.0% ± 19.0%) in the rainy season, whereas in dry season Firmicutes (83.0% ± 10.0%), mainly the class Bacilli (81.5% ± 10.0%) were the most abundant taxa (Fig. 4, 5). The phylum Nitrospirae, exclusively represented by the class Nitrospira (6.0% ± 7.0%), followed by Thaumarchaeota (6.5% ± 13.2 %), mainly the class South African Gold Mine Gp1 (SAGMCG-1; 5.8% ± 8.4 %), and Acidobacteria (4.0% ± 2.0%) were also abundant (Fig. 4, 5). Bacteroidetes, Planctomycetes, Actinobacteria, Elusimicrobia, and Verrucomicrobia were less abundant (approximately 1% of relative abundance each) (Fig. 4).

Microbiota analysis revealed a distinct taxonomic composition between water sources expressed in operational taxonomic units (OTUs) at the lowest taxonomic level possible (> 1% of the total prokaryotic community). As shown in Fig. 6, the most dominant genera in Buraco Quente water source was *Acinetobacter* (Gammaproteobacteria; 23.3% and 11.4%, in the rainy and dry seasons, respectively), followed by the two Betaproteobacteria genera *Aquabacterium* [4.0% (rainy season) and 17.0% (dry season)] and *Acidovorax* [2.2% (rainy season) and 2.5% (dry season)], *Flavobacterium* (Flavobacteriia; 9.0% and 4.5% in the rainy and dry seasons, respectively), and *Pseudomonas* [Gammaproteobacteria; 1.3% (rainy season) and 8.0% (dry season)]. Itacolomi water source was dominantly composed of an array of bacterial OTUs belonging to Betaproteobacteria, particularly (values are presented for rainy

and dry seasons, respectively): a representative OTU of the *Comamonadaceae* family (7.0% and 11.0%), *Limnohabitans* (3.6% and 9.4%), *Acidovorax* (6.6% and 3.4%), and *Aquabacterium* (1.5% and 0.5%). Of particular relevance, *Pseudarcicella* (Cytophagia) showed a relative abundance of 35% in the dry season. In Novo Horizonte water source all dominant OTUs were representatives of Betaproteobacteria, as follows: *Undibacterium* (1.7% and 34.4%), *Pelomonas* (1.1% and 3.8%), *Massilia* (3.1% and 3.1%), *Chromobacterium* (0.05% and 18.0%), and *Aquitalea* (75.0% and 1.3%), all values corresponding to the rainy and dry seasons, respectively.

In groundwater, South African Gold Mine Gp 1 (SAGMCG-1) [8.4% (rainy season) and 3.0% (dry season)] and Candidatus Nitrosotalea [10.4% (rainy season) and 2.0% (dry season)] both of them Archaea representatives prevailed. In regard to the different collection sites, the following relative abundances were observed: 1. UFOP water source - SAGMCG-1 [18.05% (rainy season) and 5% (dry season)], *Nitrospira* [(Nitrospira; 19.8% (rainy season) and 3.3% (dry season)], and specially *Geobacillus* (Bacilli; 16.7%) in the rainy season were the most dominant OTUs; 2. Mina Rainha water source - an OTU of *Oxalobacteraceae* (1.8%) and *Massilia* (12.5%), both of them included in Betaproteobacteria class, and *Pseudomonas* (Gammaproteobacteria; 3.5%) were the most abundant OTUs in the rainy season. Considering results obtained for UFOP and Mina Rainha water sources respectively, *Enterococcus* (45.8% and 37,8%), *Bacillus* (16.4% and 37.7%), and *Paenibacillus* (6.9% and 10.0%) were the most dominant OTUs in the dry season (Fig. 6).

3.4 Procaryotic taxa differentially distributed among water sources

The differential abundance analysis based on DESeq2 revealed that Firmicutes, Thaumarcheota, Nitrospirae, and Acidobacteria abundances were significantly higher in groundwater when compared with surface water. On the other hand, Bacteroidetes and Cyanobacteria showed significantly higher abundances in surface water ($p < 0.05$) (Table 2). Actinobacteria and Proteobacteria abundances were similar between both water types.

Although most OTUs were present in all water sources, the relative abundances of some of them varied according to the water source. *Bacteroides*, *Clostridium*, *Acinetobacter*, and *Flavobacterium* were particularly enriched in Buraco Quente prokaryotic community ($p < 0.05$) when compared with the other freshwater sources. In addition, many OTUs were unique to this water source, such as *Fusobacterium*, *Fusicatenibacter*, *Anaerostipes*, *Lachnoclostridium*, *Coprococcus*, *Tyzzerella*, *Blautia*, *Ruminococcus*, *Ruminiclostridium*, *Alistipes*, *Parabacteroides*, and *Sutterella*.

In regard to UFOP water source, South African Gold Mine Gp 1, Candidatus Nitrosotalea, and *Nitrospira* had greater relative abundances ($p < 0.05$) when compared with the other freshwater sources. Candidatus Nitrososphaera, Ferritrophicum, Sulfuritalea, Candidatus Methylomirabilis, and Candidatus Solibacter were some of the exclusive species found in this source. It should be mentioned that *Enterococcus* and *Bacillus* were overrepresented in groundwater sources in the dry season ($p < 0.05$) (Table 2).

3.5 Procaryotic communities and water quality parameters

The CCA multivariate analysis performed to assess the relationship between water parameters and microbial classes showed that CCA1 and CCA2 could explain 74.13 % of the total variation. The ANOVA test was used to test the model with 999 permutations ($p < 0.01$) (Fig. 7). Buraco Quente prokaryotic community was significantly correlated with COD, TS, turbidity, and conductivity. In addition, these parameters were associated with Bacteroidia, Clostridia, Gammaproteobacteria, Flavobacteriia, and Actinobacteria. Procaryotic community of groundwater sources (Mina Rainha and UFOP) in the dry season was correlated to Bacilli and showed significant negative correlation with pH and DO, whereas in the rainy season, the microbial community revealed a different profile. UFOP source was positively explained by nitrate and As, negatively explained by pH and DO, and related with South African Gold Mine Gp 1, Nitrospira, and Acidobacteria. Mina Rainha source was explained jointly with surface water sources (Itacolomi and Novo Horizonte) and positively related with ORP, pH, and DO

parameters and correlated with Betaproteobacteria, Cytophagia and Alphaproteobacteria (Fig.7).

In addition, Pearson's correlation analysis revealed significant association ($p < 0.05$) between some OTUs identified to the lowest taxonomic level possible and heavy metals. Data demonstrated the following correlations: *Sediminibacterium*, *Christensenellaceae* R.7.group, *Bosea*, *Massilia*, and *Gemmata* with Fe; *Anoxybacillus* with Ni; *Stenotrophomonas*, *Aeromonas*, and *Flavobacterium* with Mn; *Arthrobacter*, *Burkholderia*, *Lysinibacillus*, representative OTU of *Planococcaceae*, *Bdellovibrio*, *Nitrospira*, *Opitutus*, SAGMCG-1 and *Brevundimonas* with As; *Ralstonia*, representative OTU of *Rhizobiaceae*, *Desulfovibrio*, and Candidatus *Captivus* with As and Fe; *Acinetobacter*, representative OTU of *Enterococcaceae*, and representative OTU of Lactobacillales with Mn and Ni; and *Enterobacter* and *Staphylococcus* with Mn and As. Some bacteria were strongly positively associated to three metals, Ni, Mn, and As. They are *Alkaliphus*, *Bacillus*, *Enterococcus*, *Oceanobacillus*, *Planomicrobium*, *Sporosarcina*, and a representative OTU of Bacilli (Table 3).

3.6 Pathogenic bacteria and indicators of water quality

Bacterial genera known to include important potential waterborne pathogens and fecal indicators were detected in freshwater sources, as shown in Figure 8. Sequences matching potential pathogens found in all water samples both groundwater and surface water, but generally with a relative abundance less than 1% included *Escherichia-Shigella*, *Aeromonas*, *Arcobacter*, *Mycobacterium*, *Acinetobacter*, *Pseudomonas*, *Clostridium*, *Enterococcus*, *Enterobacter*, *Rickettsia*, *Bacillus*, *Staphylococcus*, *Methylobacterium*, *Coxiella*, *Legionella*, *Streptococcus*, *Staphylococcus*, *Mycoplasma*, *Corynebacterium*, *Burkholderia*, *Vibrio*, *Salmonella*, *Serratia*, *Moraxella*, *Aerococcus*, *Streptococcus*, *Klebsiella*, and *Neisseria* (Fig. 8).

In Buraco Quente water source, an increase in the relative abundance of several bacteria, including *Arcobacter*, *Aeromonas*, *Pseudomonas*, *Klebsiella*, *Streptococcus*, *Enterobacter*,

Legionella, *Clostridium*, *Moraxella*, *Salmonella*, and *Bacillus*, besides those considered as fecal indicators, *Escherichia-Shigella*, *Prevotella*, *Blautia*, and *Enterococcus*, was observed in both seasons. Novo Horizonte provides freshwater with lower relative abundance of these genera when compared to the other water sources (Fig. 8).

According to PATRIC database, it was determined that *Acinetobacter* sequences were attributed to *Acinetobacter baumannii* (ID1310571.3.rna.49); *Pseudomonas* sequences were attributed to *Pseudomonas putida* (ID 390235.5.rna.8), *Pseudomonas fluorescens* (ID216595.4.rna.4), and *P. aeruginosa* (ID 1365011.3.rna.50); *Aeromonas* sequences were classified as *Aeromonas caviae* (ID 648.88.rna.8), *Aeromonas simiae* (ID218936.3rna.68), and *A. veronii* (ID654.94.rna.5); *Klebsiella* sequences were attributed to *Klebsiella oxytoca* (ID883117.3.rna.39); *Bacillus*-related OTUs were classified as *Bacillus cereus* (ID526977.3.rna.92); OTUs affiliated to *Enterococcus* were classified as *E. faecium* (ID565663.4.rna.55) and *E. faecalis* (ID565646.5.rna.35); *Escherichia-Shigella* sequences were classified as *E. coli* (ID 585057.rna.67); and *Mycobacterium* sequences were attributed to *Mycobacterium intracellulare* (ID 1299331.3.rna.14). The other sequences classified at the genus level could not be identified to the species level.

4. Discussion

Water quality is an emergent property of a complex system comprised of interacting microbial populations and introduced microbial and chemical contaminants. Studies using next-generation sequencing technologies are continuously providing new insights into the microbial ecology that influence drinking water sources quality in several regions of the world (Tan et al., 2015). Thus, we aimed at contributing to the understanding of the microbial diversity of freshwater resources from an important touristic city in Brazil.

Drinking water sources show a high microbial diversity according to literature data. In fact, freshwater ecosystem is one of the natural environments that displays the richest microbial diversity (Tamames et al., 2010). Nevertheless, some differences in procaryotic diversity were

observed between groundwater and surface water sources. Alpha- and beta-diversity analyses revealed that groundwater, but not surface water is subjected to seasonal changes. In the rainy season the richness and diversity measurements of groundwater were higher than in the dry season and the bacterial community was also different (Fig. 2, 3). These observations may be explained by the fact that the prokaryotic community may be affected by several other physical variables other than rain, such as soil, vegetal cover, residual contamination, and minerals that influence more markedly surface water than groundwater. Groundwater is more dependent on rain because its volume is replenished by precipitation. In this way, water infiltrates into the pores or cracks of the soil and rocks and accumulates, forming the groundwater and increasing microbial diversity (Winter et al.,1998; Moreira and Bondelind, 2017). Major aquatic environments such as surface water and groundwater form a discrete ecological unit, with their own characteristic community of organisms and each contains distinctive groups of microorganisms (Sigee, 2005) (Fig. 3).

Procaryotic communities of surface water in both seasons and of groundwater in the rainy season showed to be dominantly composed of Proteobacteria (Fig. 4). This result corroborates previously reported data that demonstrate that the phylum generally predominates in aquatic ecosystems (Karwautz and Lueders, 2014, Elchkhtoura et al., 2015). In addition, the present study also confirms Betaproteobacteria as the dominant class in such environments (Fig. 5) (Emtiazi et al., 2004; Lautenschlager et al., 2013; Elchakhtoura et al., 2015, Hassan et al., 2015). Gammaproteobacteria and Alphaproteobacteria also may be abundant in freshwater (Hwang et al., 2012; Chao et al., 2013) as demonstrated by the present investigation (Fig. 5). The major Proteobacteria representatives included *Acinetobacter*, *Limnohabitans*, *Acidovorax*, *Pseudomonas*, *Undibacterium*, *Pelomonas*, *Aquitalea*, *Chromobacterium*, *Massilia*, and a representative OTU of *Oxalobacteriaceae* (Fig. 6). These bacteria are considered as the most widespread ones in soil and freshwater (Newton et al., 2011).

In contrast, the groundwater sources microbioma in the dry season were dominantly composed of phylum Firmicutes, particularly class Bacilli, and *Enterococcus* and *Bacillus* genera (Fig. 4, 5, 6). This result contrasts with most literature data that shows that this phylum is detected in

a proportion close to 5% in freshwater (Elchakhtoura et al., 2015, Hassan et al., 2015). A similar result was reported by Mukherjee et al. (2016) that by using 16S rRNA gene V4 region sequencing, found the predominance of Firmicutes in drinking water from rural sources in Haiti, with *Bacillus* and *Clostridium* as the dominant genera. Firmicutes has also been reported as the dominant phylum in wastewater samples with an elevated level of pollution and extreme conditions (Wang et al., 2014).

According to PATRIC BLASTn queries, *Bacillus*-related OTUs were classified as *B. cereus*. The species is commonly found in a wide range of natural environments, such as soil (Radhakrishnam et al., 2017). In turn, OTUs affiliated to *Enterococcus* genus were classified by PATRIC database as *E. faecium* and *E. faecalis.* Although *Enterococcus* species presence provides evidence of late fecal contamination, they may also be found in soil and water environments (Byappanahalli et al., 2012), what may justify the absence of correlation between *Enterococcus* and water samples presenting parameters frequently related to fecal contamination. The presence of Bacilli was negatively influenced by pH and DO (Fig. 7), possibly due to *Enterococcus* and *Bacillus* resistance to acidic environments and low concentrations of oxygen, characteristics of groundwater drained with mining activities (Krulwich et al.,1985; Byappanahalli et al., 2012).

Thaumarchaeota, Nitrospira, and Acidobacteria were also abundant in groundwater showing a significantly higher relative abundance in this kind of water source (Fig. 4, Table 2). Thaumarchaeota are considered prominent members of Archaea and commonly found in groundwater sources, providing further evidence of the versatility and cosmopolitan nature of the domain in the environment (Biller et al., 2012). Among OTUs included in this phylum, South African Gold Mine Gp 1 (SAGMCG-1) and Candidatus Nitrosotalea should be highlighted. SAGMCG-1 is a novel archaeal member that specifically inhabit deep terrestrial subsurface environments and were first recovered from deep South African gold mine environments (Takai et al., 2001), similar to Ouro Preto groundwater environment which is also influenced by gold mining activities. Other Archaea representative Candidatus Nitrosotalea is abundant and widely distributed in acidic soils globally (Gubry-Rangin et al., 2011). Nitrospira and

415 Acidobacteria are widespread and abundant on soil and aquatic ecosystems and are
416 frequently recovered from groundwater (Koch et al., 2015; Kielak et al., 2016).
417 South African Gold Mine, Nitrospira, and Acidobacteria were all associated with UFOP
418 groundwater source in the rainy season. They were positively correlated with nitrate and As
419 and negatively associated with pH and DO (Fig. 7). SAGMCG-1 and Candidatus Nitrosotalea
420 have also been recognized as abundant organisms that contribute to ammonia oxidation in the
421 majority of terrestrial and aquatic habitats (Lehtovirta-Morley, 2016). Nitrospira is generally
422 found in aquatic environments and plays a role in the nitrogen cycle, by performing nitrite
423 oxidation (Lucker et al., 2010). These bacteria often occur in close association to convert
424 ammonia to nitrite and nitrite to nitrate (Daebeler et al., 2014), thus involved in several stages
425 of nitrogen metabolism. Some OTUs were unique to UFOP water source, such as Candidatus
426 Nitrososphaera, Candidatus Solibacter, and Candidatus Methylomirabilis (Table 2). They are
427 also involved in nitrogen cycle, suggesting that this element may play an important role in the
428 trophic chain of this groundwater microbial community (Tetu et al., 2013). *Ferritrophicum* and
429 *Sulfuritalea* were also exclusive OTUs (Table 2), frequently reported in mine-impacted water
430 bodies and related to ammonium-oxidizing bacteria (Weiss et al., 2007, Mühling et al., 2016).
431 Bacteroidetes and Cyanobacteria had a significant higher abundance in surface water when
432 compared to groundwater (Table 2). Indeed, Bacteroidetes is known to be the most prominent
433 heterotrophic organisms in surface water sources (Steven et al., 2005) and Cyanobacteria are
434 photosynthetic organisms that require light to convert water and carbon dioxide to
435 carbohydrates and oxygen, explaining its association with surface water (Kim, 2017).
436 Buraco Quente surface water source bacterial community was significantly correlated to COD,
437 TS, turbidity, and conductivity. All of these indexes found in this water source are above the
438 maximum permissible level according to Brazilian legislation (Fig. 7, S1). In addition, these
439 parameters were correlated with Bacteroidia, Clostridia, Gammaproteobacteria, Flavobacteria,
440 and Actinobacteria classes (Fig. 7). Among them, Bacteroidia and Clostridia are taken as
441 alternative indicators of fecal contamination in water (Yang et al., 2011; Shahryari et al., 2015).
442 Gammaproteobacteria, including *Acinetobacter*, *Pseudomonas* and *Enterobacteriaceae*,

443 comprises an important group of bacteria frequently associated to human impacted
444 environments (Williams et al., 2010; Xia et al., 2010; Ye and Zhang, 2012). In regard to
445 Flavobacteria and Actinobacteria, both of them play a relevant role in the decomposition of
446 organic matter and carbon cycle (Abell and Bowman, 2005; Lewin et al., 2016). Unique OTUs
447 related to Buraco Quente (Table 2), such as *Fusobacterium*, *Parabacteroides*, and
448 *Ruminococcus* are mostly bacteria considered as members of the intestinal microbiota of
449 humans and other animals (Stojanovic and de Vos, 2014). Taken together, these results reveal
450 that Buraco Quente water source presented inadequate water quality parameters both in the
451 rainy and dry seasons indicating contamination by human activity, animal feces, or sewage.
452 Data clearly demonstrate that this water source is unsuitable for consumption, and should not
453 continue to be used as an alternative drinking water source by the population.
454 Water sources bacterial community at the OTU level was correlated with heavy metals (Ni, Fe,
455 As, and Mn) (Table 3). Considering that heavy metals are ubiquitously present in aquatic
456 environments, microorganisms that inhabit such habitats frequently express mechanisms that
457 confer resistance to their toxic effects (Ayangbenro and Babalola, 2017). *Bacillus*,
458 *Acinetobacter*, *Ralstonia*, *Staphylococcus*, *Burkholderia*, and *Enterobacter*, which are
459 frequently reported to be resistant to metals (Oremland et al., 2004; Rajbanshi, 2008) were
460 identified in strong association with heavy metals in the present study. *Sediminibacterium*,
461 *Flavobacterium*, and *Nistrospira* have been previously reported as predominant genera in
462 sediments of freshwater contaminated by heavy metals in the region of Ouro Preto (Costa et
463 al., 2015). In this study, several genera of Firmicutes were found to be correlated with various
464 heavy metals, such as *Bacillus*, *Enterococcus*, *Oceanobacillus*, *Paenibacillus*,
465 *Planomicrobium*, *Sporosarcina*, a representative OTU of Bacilli, *Enterococcaceae*, and
466 Lactobacillales, and *Lysinibacillus* (Table 3). Interestingly, *Enterococcus* was correlated with
467 three metals and it is plausible to hypothesize that this may be related to its frequent
468 multiresistance to antibiotics. A frequent association between tolerance to heavy metals and
469 antimicrobial resistance has already been reported (Pal et al., 2017).

The major diseases related to contaminated drinking water are caused by enteropathogenic bacteria. In addition to these classical waterborne pathogens, there are many environmental pathogens, often opportunistic, also vehiculated by water (Ashbolt, 2015). Sequences matching potential pathogens were found in both groundwater and surface water used for public and alternative water supply but their relative abundance was generally less than 1% (Fig. 8). *A. baumannii*, *P. aeruginosa*, *P. fluorescens, P. putida*, *A. caviae*, *A. simiae*, *Aeromonas veronii*, *M. intracellulare*, *Methylobacterium*, *Staphylococcus*, *Serratia*, *Moraxella*, *Legionella* detected are considered environmental pathogens often opportunistic (Ashbolt, 2015). *K. oxytoca, Arcobacter*, *E. faecium*, *E. faecalis*, and *Enterobacter* are also opportunistic pathogens found in the environment but also in the human intestinal tract (Collado and Figueras, 2011; Castillo-Rojas et al., 2013; Davin-Regli and Pagés, 2015; Singh et al., 2016). *E. coli*, *Salmonella*, *Bacillus cereus*, and *Clostridium* are classical waterborne pathogens that can cause diarrheal disease or food poisoning (Ashbolt, 2015).

Increased relative abundance of several bacteria, including *Arcobacter*, *Aeromonas*, *Pseudomonas*, *Klebsiella*, *Streptococcus*, *Enterobacter*, *Legionella*, *Clostridium*, *Moraxella*, *Salmonella* and *Bacillus*, besides those related to fecal indicators, *Escherichia-Shigella*, *Prevotella*, *Blautia* and *Enterococcus*, was observed in Buraco Quente. In contrast, Novo Horizonte which is also an alternative surface water source provides water with superior microbiological quality considering the lower abundances of the these OTUs (Fig. 8).

It should be emphasized that the detection of these OTUs do not confirm the presence of viable pathogenic bacteria. Despite this limitation inherent to the methodology employed, the diversity of potentially pathogenic bacteria found in the water samples raises concern in regard to public health. Molecular genetic tools are taken as effective means for monitoring very diverse biological targets. In a near future at more affordable costs, they have the potential to significantly improve microbial risk assessment and management capacities in freshwater sources used as drinking water (Ramirez-Castillo et al., 2015).

5. Conclusions

This study provided a 16S rRNA gene deep sequencing analysis view of the procaryotic community structure and diversity of groundwater and surface water sources used to feed DWTS in Ouro Preto or employed as alternative water sources by the population of the city. The study revealed seasonal variations in prokaryotic community structure and diversity of groundwater sources. Enriched and differentiated communities may reveal the profile of the freshwater source, as seen in Buraco Quente water source (surface water) that showed a disturbed prokaryotic community consequently to fecal contamination, and in the groundwater sources that revealed the dominance of microorganisms adapted to acidic, low oxygen concentration, and high nitrate and arsenic conditions. Multiple associations were observed between several OTUs and heavy metals, what may suggest further studies to evaluate the potential of these prokaryotic organisms for bioremediation of water and sediments contaminated with metals. Also considering that some of these genera show clinical relevance and not rare express antimicrobial resistance the results may indicate an association between resistance to antibiotics and heavy metals. The detection of pathogenic bacteria in freshwater sources alerts to the risks associated with the use of these water sources, particularly those employed as alternative sources that are consumed without any adequate treatment.

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Figure1. Geographical location of drinking water sources in Ouro Preto, Minas Gerais, Brazil.

Table 1. Drinking water sources description.

Name	Abbreviation	Water type	Supply system	Latitude	Longitude
Buraco Quente	BQ	Surface water	Alternative	20.23.10º	43.31.01º
Itacolomi	IT	Surface water	Public	20.24.20º	43.29.53º
Mina Rainha	MR	Groundwater (abandoned gold mines)	Public	20.22.38º	43.30.59º
Novo Horizonte	NH	Surface water	Alternative	20.24.15º	43.30.00º
UFOP	UF	Groundwater (tubular well)	Alternative	20.23.42º	43.30.47º

UFOP, Universidade Federal de Ouro Preto.

Supplementary 1. Description of physicochemical properties of drinking water samples.

Sample	Temperature (°C)	pH	Turbidity (NTU)	TS (mg/L)	DO (mg/L)	Ammonia (mg/L)	Nitrate (mg/L as NO_3^-)	Nitrite (mg/L as NO_2^-)	Phosphate (mg/L as PO_4^{3-})	BOD (mg/L)	COD (mg/L)	ORP (mV)	Conductivity (µS/cm)	As (mg/L)	Mn (mg/L)	Ni (mg/L)	Fe (mg/L)
BQr	24.5	7.4	3.23	0.02	7.4	3.36	2.6	0.060	0.31	3.09	175.00	252.0	77.78	0.0000	0.547	0.10	0.400
BQd	24.0	6.9	3.00	0.10	7.4	2.80	3.4	0.117	0.3	7.21	102.00	170.0	97.30	0.0000	0.257	0.11	0.062
ITr	19.5	7.7	1.32	0.06	8.0	6.00	2.0	0.005	0.15	0.04	75.0	680.0	16.42	0.0070	0.000	0.10	0.080
ITd	18.5	7.0	1.00	0.02	8.64	1.68	1.9	0.004	0.18	0.90	1.35	709.0	41.52	0.0000	0.050	0.11	0.013
MRr	20.8	5.4	1.00	0.02	7.72	7.00	2.7	0.002	0.24	0.20	65.00	533.0	18.05	0.0270	0.000	0.10	0.000
MRd	18.9	5.4	1.00	0.02	7.7	3.36	1.6	0.002	0.2	0.19	0.32	126.0	62.79	0.0060	0.130	0.11	0.274
NHr	22.5	7.0	0.04	0.01	7.5	9.00	2.6	0.003	0.22	0.05	40.00	316.0	12.51	0.0000	0.360	0.10	0.000
NHd	21.0	7.0	0.04	0.02	8.0	8.00	2.1	0.003	0.2	0.59	0.78	222.0	45.83	0.0000	0.040	0.11	0.053
UFr	22.2	6.2	0.04	0.02	6.42	0.12	3.6	0.002	0.28	0.14	40.00	254.0	46.83	0.0710	0.090	0.10	0.000
UFd	22.4	5.0	0.04	0.02	7.0	0.12	3.9	0.003	0.28	0.52	0.78	25.2	39.74	0.0015	0.110	0.12	0.068

Physicochemical parameters - TS: total solids, DO: dissolved oxygen, BOD: biochemical oxygen demand, COD: chemical oxygen demand, ORP: oxidation reduction potential, As: arsenic, Mn: manganese, Ni: nickel, Fe: iron. Sample - BQ: Buraco Quente (surface water source), IT: Itacolomi (surface water source), MR: Mina Rainha (groundwater source), NH: Novo Horizonte (surface water source), UF: UFOP (Universidade Federal de Ouro Preto; groundwater source), r: rainy season, d: dry season.

Supplementary 2. Number of reads generated on MiSeq platform and after contig assembly, processing, and filtering (postprocessed).

Sample	Generated reads	Postprocessed reads
BQr	137,596	80,180
BQd	145,751	91,733
ITr	141,177	71,265
ITd	125,547	64,422
MRr	67,904	32,433
MRd	47,558	25,342
NHr	106,713	94,616
NHd	99,734	78,124
UFr	66,135	35,290
UFd	45,653	28,634
Total	983,768	602,039

BQ: Buraco Quente (surface water source), IT: Itacolomi (surface water source), MR: Mina Rainha (groundwater source), NH: Novo Horizonte (surface water source), UF: UFOP (Universidade Federal de Ouro Preto; groundwater source), r: rainy season, d: dry season.

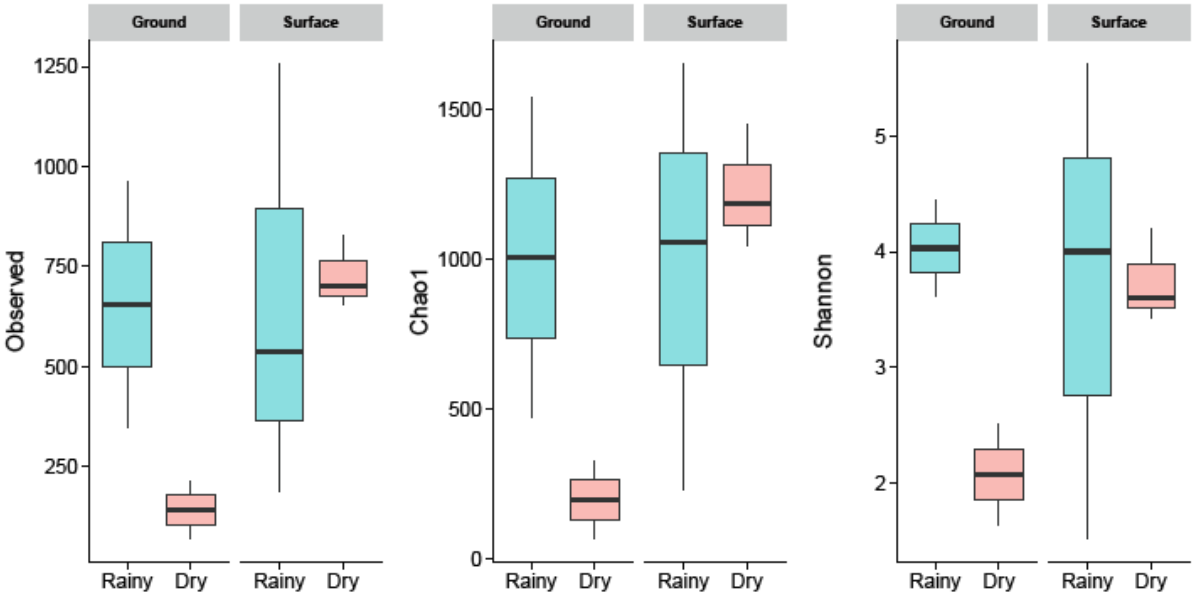

Figure 2. Alfa-diversity of the procaryotic community of groundwater and surface water sources in the rainy and dry seasons expressed as the number of observed OTUs, Chao1, and Shannon indexes. Boxes represent the interquartile range and the line inside represents the median.

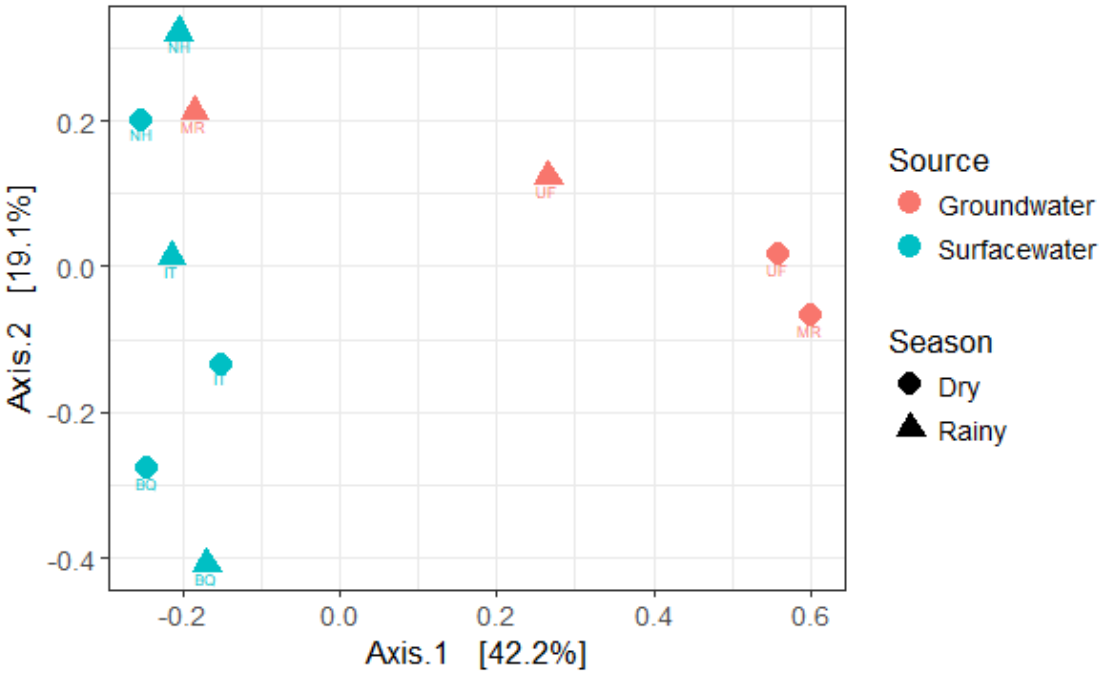

Figure 3. Beta-diversity of the procaryotic community of groundwater and surface water in the rainy and dry seasons based on unweighted UniFrac distance matrix using principal coordinates analysis (PCoA). BQ: Buraco Quente (surface water source), IT: Itacolomi (surface water source), MR: Mina Rainha (groundwater source), NH: Novo Horizonte (surface water source), UF: UFOP (Universidade Federal de Ouro Preto; groundwater source).

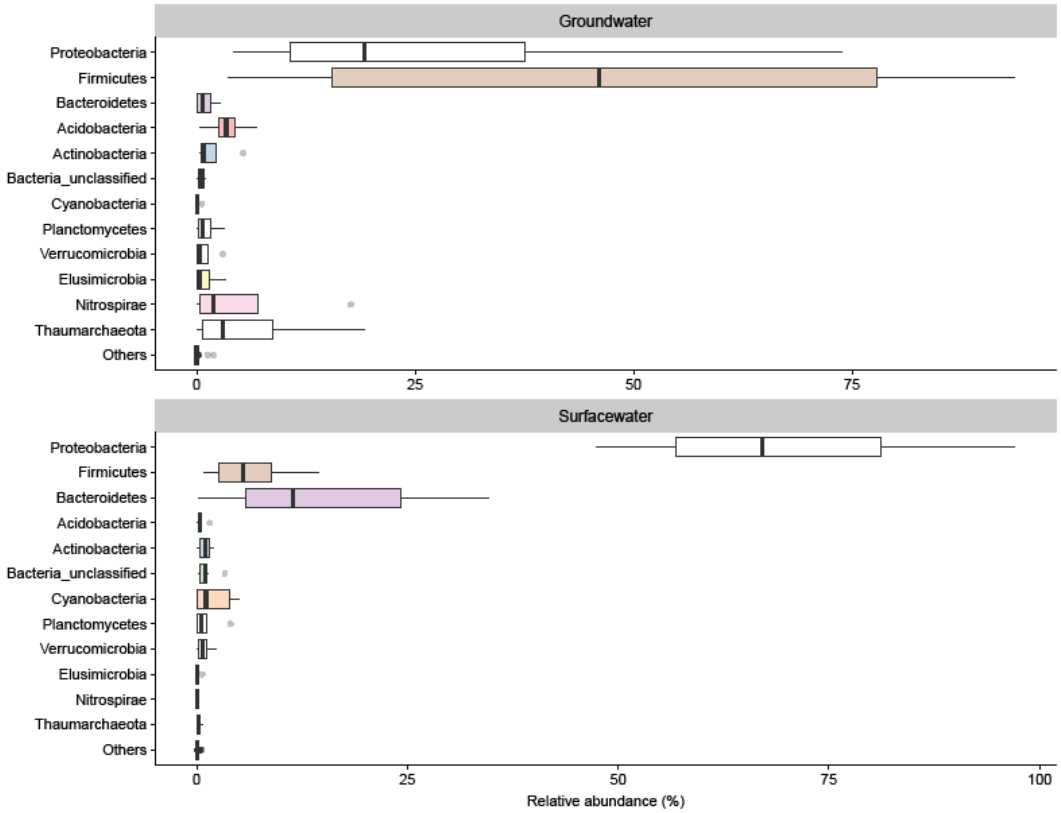

Figure 4. Taxonomic profile of the groundwater and surface water sources according to phyla abundance. The twelve most abundant phyla are shown and other phyla are presented as “others”. Boxes represent the interquartile range and the line inside represents the median.

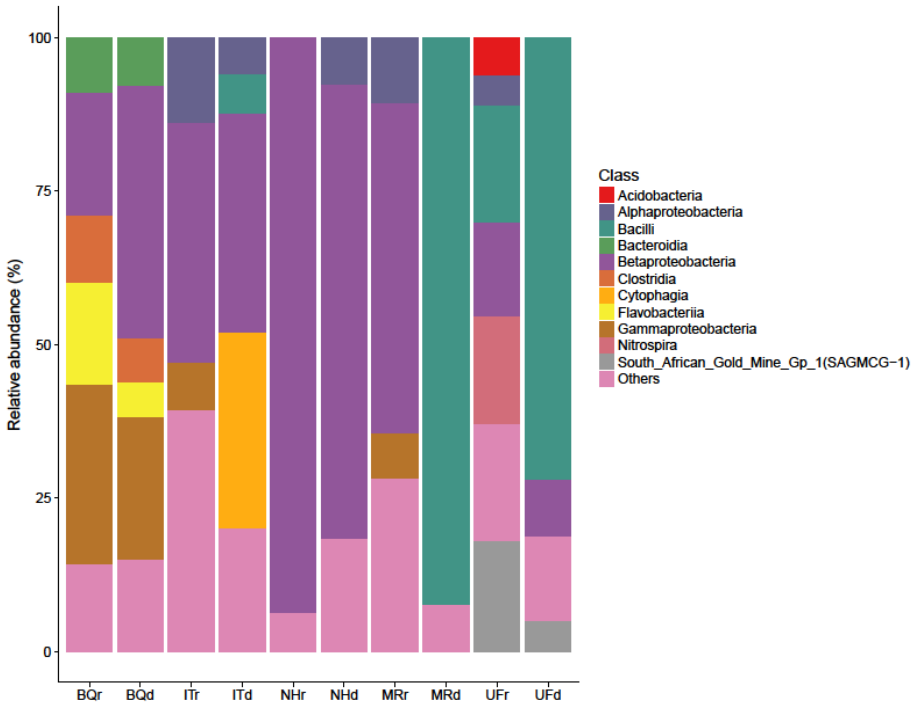

Figure 5. Relative abundance of the eleven most abundant classes of groundwater and surface water sources. BQ: Buraco Quente (surface water source), IT: Itacolomi (surface water source), NH: Novo Horizonte (surface water source), MR: Mina Rainha (groundwater source), UF: UFOP (Universidade Federal de Ouro Preto; groundwater source), r: rainy season, d: dry season.

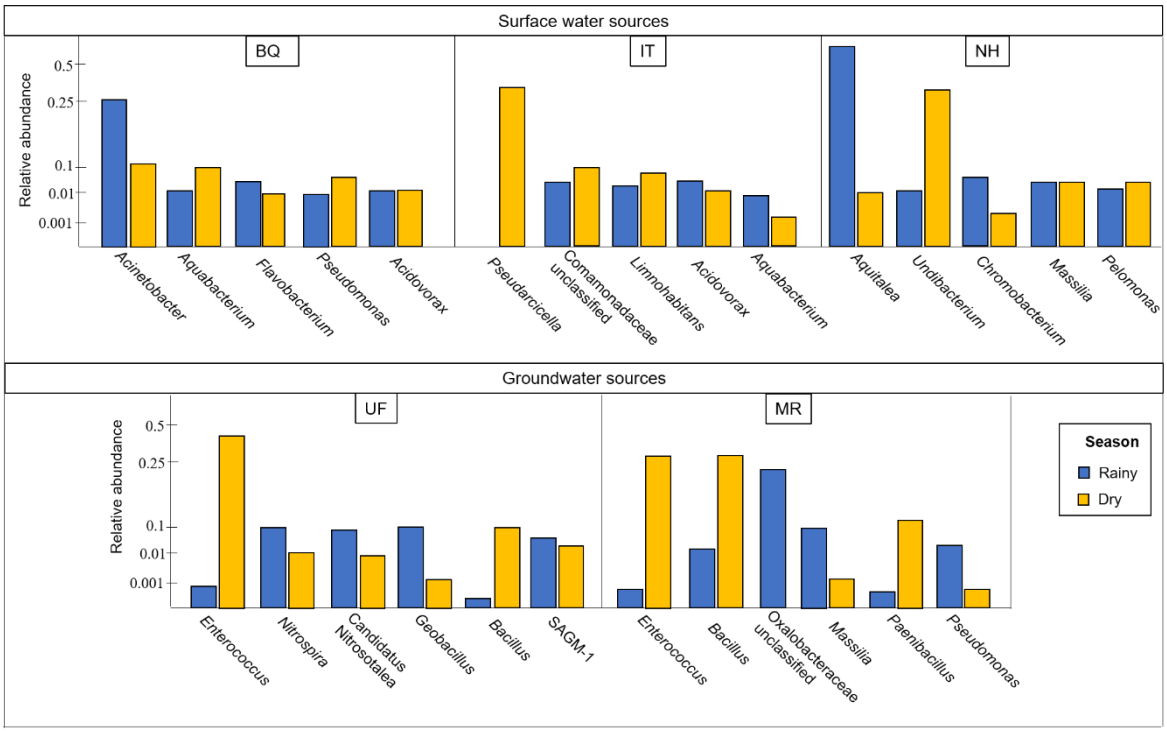

Figure 6. Relative abundance of the most abundant operational taxonomic units (OTUs) identified at the lowest taxonomic level possible of groundwater and surface water sources. BQ: Buraco Quente (surface water source), IT: Itacolomi (surface water source), NH: Novo Horizonte (surface water source), UF: UFOP (Universidade Federal de Ouro Preto; groundwater source), MR: Mina Rainha (groundwater source).

Table 2. Exclusive OTUs and taxa with different abundance* based on DESeq2.

Source	Phyla	OTUs	Exclusive OTUs
Surface water	Bacteroidetes Cyanobacteria	*Bacteroides* *Clostridium* *Acinetobacter* *Flavobacterium* (in BQ)	*Fusobacterium* *Fusicatenibacter* *Anaerostipes* *Lachnoclostridium* *Coprococcus* *Tyzzerella* *Blautia* *Ruminococcus* *Ruminiclostridium* *Alistipes* *Parabacteroides* *Sutterella* (in BQ)
Ground water	Firmicutes Thaumarcheota Nitrospirae Acidobacteria	South African Gold Mine Gp 1 (SAGMCG-1) Candidatus Nitrosotalea *Nitrospira* (in UF) *Enterococcus* *Bacillus* (in DS)	Candidatus Nitrososphaera *Ferritrophicum* *Sulfuritalea* Candidatus Methylomirabilis Candidatus Solibacter

$p < 0.05$; BQ, Buraco Quente; UF, UFOP; DS, dry season.

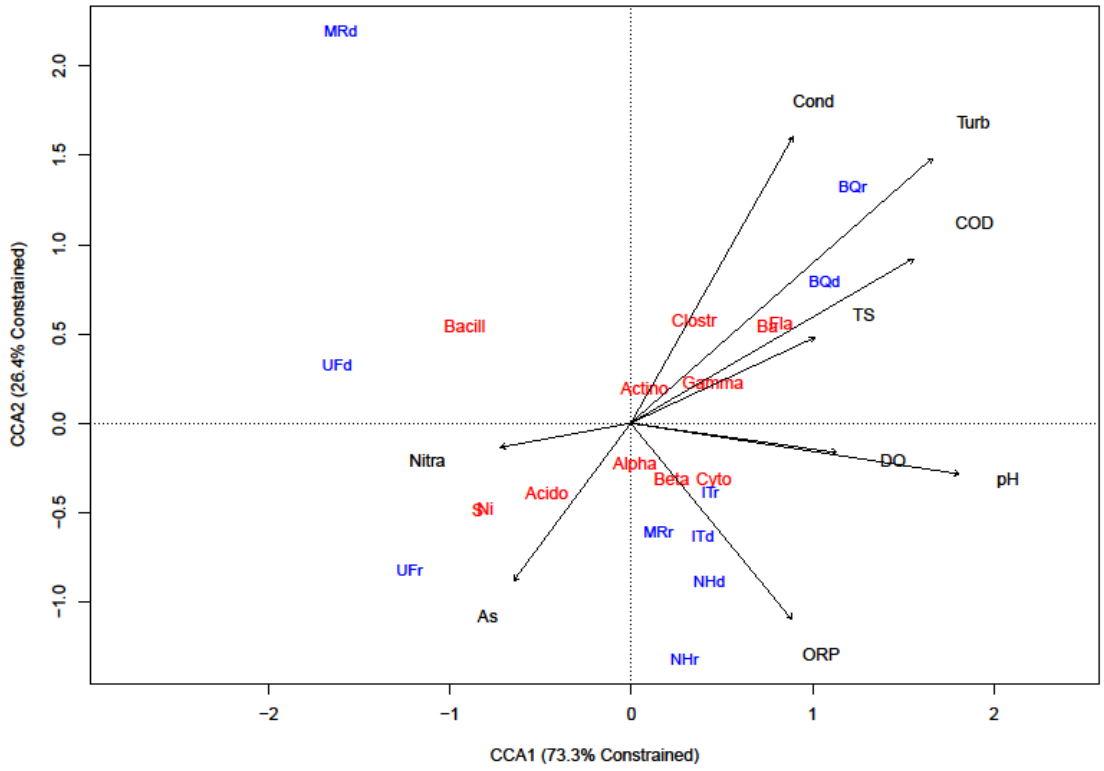

Figure 7. Canonical correlation analysis of sources water prokaryotic communities at class level in groundwater (MR and UF) and surface water (BQ, IT, and NH). CCA1 explained 73.3 %, and RDA2 explained 26.4 % of the total variance.

Physicochemical parameters: TS, total solids; DO, dissolved oxygen; COD, chemical oxygen demand; ORP, oxidation reduction potential; As, arsenic; Nitra, nitrate; Cond, conductivity; Turb, turbidity.

Abbreviations of classes names: Bacill, Bacilli; Alpha, Alphaproteobacteria; Beta, Betaproteobacteria; Gamma, Gammaproteobacteria; Acido, Acidobacteria; Cyto, Cytophagia; Actino, Actinobacteria; Clostr, Clostridia; Ba, Bacteroides; Fla, Flavobacteriia. The ANOVA (permutation = 999) was used to test the model with $p < 0.05$.

Table 3. Pearson's correlation coefficient between heavy metals and relative abundance of genera and OTU.

Taxon	Iron	Nickel	Manganese	Arsenic
Ralstonia	0.49	-	-	0.59
Sediminibacterium	0.58	-	-	-
unclassified_*Rhizobiaceae*	0.42	-	-	0.56
Christensenellaceae R.7.group	0.43	-	-	-
Bosea	0.52	-	-	-
Desulfovibrio	0.51	-	-	0.50
Massilia	0.58	-	-	-
Candidatus *Captivus*	0.44	-	-	0.48
Gemmata	0.52	-	-	-
Alkaliphilus	-	0.62	0.64	0.62
Acinetobacter	-	0.66	0.75	-
Anoxybacillus	-	0.64	-	-
Bacillus	-	0.43	0.47	0.57
Enterococcus	-	0.53	0.55	0.55
Oceanobacillus	-	0.48	0.49	0.47
Paenibacillus	-	0.5	-	0.57
Planomicrobium	-	0.53	0.51	0.44
Sporosarcina	-	0.43	0.45	0.62
unclassified_Bacilli	-	0.51	0.68	0.44
unclassified_*Enterococcaceae*	-	0.56	0.50	-
unclassified_Lactobacillales	-	0.67	0.60	-
Enterobacter	-	0.00	0.64	0.67
Stenotrophomonas	-	0.00	0.73	0.00
Aeromonas	-	-	0.46	-

Flavobacterium	-	-	0.55	-
Staphylococcus	-	-	0.54	0.49
Arthrobacter	-	-	-	0.55
Burkholderia	-	-	-	0.53
Lysinibacillus	-	-	-	0.54
unclassified_*Planococcaceae*	-	-	-	0.52
Bdellovibrio	-	-	-	0.53
Nitrospira	-	-	-	0.52
Opitutus	-	-	-	0.52
SAGMCG-1	-	-	-	0.48
Brevundimonas	-	-	-	0.49

$p < 0.05$.

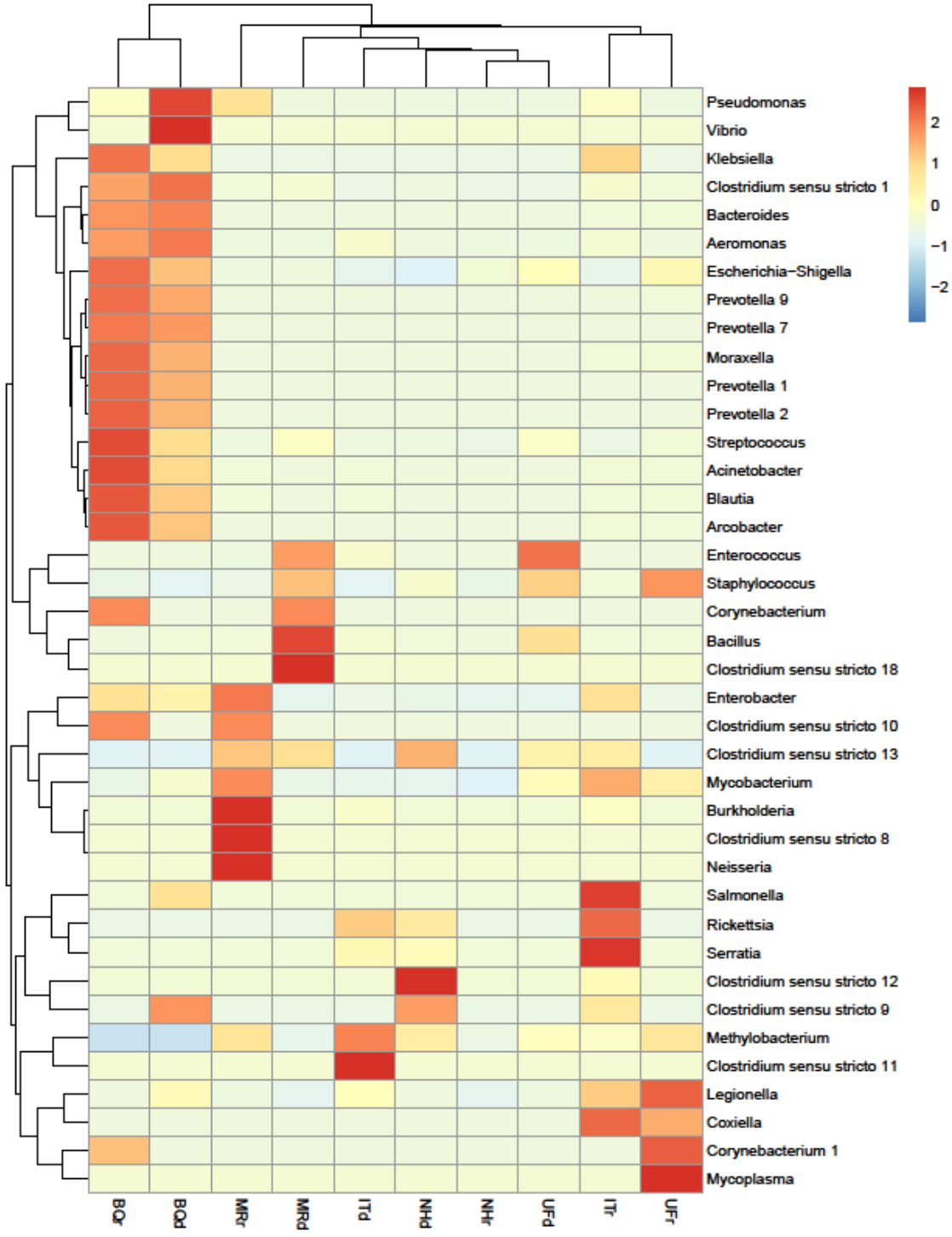

Figure 8. Heat map of potentially pathogenic bacteria community composition at genus level compared by color gradients among groundwater and surface water sources. BQ: Buraco Quente (surface water source), MR: Mina Rainha (groundwater source), IT: Itacolomi (surface water source), NH: Novo Horizonte (surface water source), UF: UFOP (Universidade Federal de Ouro Preto; groundwater source).

5 DISCUSSÃO

Águas doces superficiais e subterrâneas são importantes ecossistemas aquáticos que abrigam uma complexa diversidade microbiana e são utilizadas como fonte de água potável. Entretanto, nas últimas décadas, o aumento do tamanho da população humana e a urbanização exerceram uma imensa pressão sobre o uso desses recursos hídricos para a o consumo humano (TAN *et al.*, 2015). A contaminação por esgoto, nutrientes e, como consequência, a proliferação de microrganismos, podem tornar a água imprópria para consumo humano. O monitoramento biológico usando espécies indicadoras é frequentemente usado pelas autoridades de gestão da área, para inferir a qualidade da água, com o intuito de proteger a população de riscos de veiculação hídrica (CAREW *et al.*, 2013). Entretanto, avanços em métodos de genética molecular, em especial, sequenciamento de alto rendimento do rDNA 16S, trouxeram novas oportunidades para a análise da diversidade microbiana presente, além dos indicadores comumente investigados, fornecendo novos conhecimentos sobre a ecologia de processos mediados por microrganismos que influenciam a qualidade da água doce (TAN *et al.*, 2015; PREST *et al.*, 2016).

As fontes de água para consumo humano de Ouro Preto exibiram uma alta diversidade procariótica, de acordo com os índices de alfa-diversidade, o que ratifica a literatura, que demonstra que o *habitat* água doce alberga uma enorme diversidade microbiana, destacando-se entre os ambientes naturais (TAMAMES *et al.*, 2010). As fontes de água superficial demonstraram a presença de comunidades procarióticas similares nas estações seca e chuvosa. Entretanto, no geral, quando comparadas a águas subterrâneas, diferenças foram observadas. A diversidade e a estrutura das comunidades procarióticas das fontes de águas subterrâneas indicaram influência da chuva; no período chuvoso, os índices de diversidade e riqueza aumentaram significativamente nestes ambientes. A explicação para o achado pode envolver o fato de que as comunidades procarióticas das águas superficiais, ao contrário das águas subterrâneas, podem ser afetadas, de maneira importante, por outras numerosas variáveis físicas além da chuva, como solo, cobertura vegetal, contaminação residual e presença de minerais. A água subterrânea é mais dependente da chuva, porque é reabastecida pela precipitação.

A água se infiltra nos poros ou fissuras do solo e das rochas e se acumula, formando as águas subterrâneas, consequentemente, aumentando a sua diversidade (WINTER *et al.*, 1998; MOREIRA; BONDELIND, 2017).

A comunidade procariótica autóctone das fontes da região foi composta, dominantemente, pelo filo Proteobacteria. Este resultado reforça aqueles obtidos em diversos outros trabalhos que avaliaram água doce (KARWAUTZ; LUEDERS, 2014; ELCHAKHTOURA *et al.*, 2015) e, no geral, este filo tem predominado em ecossistemas aquáticos dulcícolas. Adicionalmente, o presente estudo corrobora estudos anteriores que reportaram Betaproteobacteria como a classe predominante nesses ambientes (EMTIAZI *et al.*, 2004; LAUTENSCHLAGER *et al.*, 2013; ELCHAKHTOURA *et al.*, 2015, HASSAN *et al.*, 2015), enquanto Alphaproteobacteria e Gammaproteobacteria foram menos abundantes. As OTUs com maior abundância representantes desse filo foram *Aquitalea, Undibacterium*, *Acinetobacter, Massilia*, *Aquabacterium, Oxalobacteraceae* não classificada, *Comamonadaceae* não classificada, *Pseudomonas*, *Pelomonas*, *Acidovorax*, *Chromobacterium* e *Limnohabitans*. De fato, esses táxons bacterianos são extensamente difundidos no solo e em ambientes de água doce (NEWTON, 2011).

Em contraste, as águas subterrâneas, na época de seca, mostraram uma predominância do Filo Firmicutes, particularmente classe Bacilli e as espécies *Enterococcus faecalis*, *Enterococcus faecium* e *Bacillus cereus* foram as mais abundantes. Este resultado diverge da maior parte dos dados disponíveis na literatura, que apontam detecção do filo em água doce em proporção próxima de 5% (ELCHAKHTOURA *et al.,* 2015, HASSAN *et al.,* 2015). O que pode justificar a dominância desses táxons bacterianos nessas fontes é que bactérias do filo Firmicutes têm sido reportadas como dominantes em ambientes com condições extremas (WANG *et al.*, 2014), como as encontradas nas fontes de águas subterrâneas, com baixo pH e oxigênio dissolvido. Embora a presença de *E. faecalis* e *E. faecium* forneça evidências de contaminação fecal, essas bactérias também podem ser encontradas em ambientes de solo e água e resistir às condições de pH e oxigênio dissolvido observadas (BYAPPANAHALLI *et al.*, 2012).

As classes Bacteroidia, Clostridia, Gammaproteobacteria, Flavobacteriia e Actinobacteria foram positivamente correlacionadas aos parâmetros de poluição orgânica (demanda química de oxigênio, sólidos totais, turbidez e condutividade).

De fato, esses táxons são frequentemente associados com ambientes impactados pelo homem. As classes Bacteroidia e Clostridia incluem, inclusive, bactérias usadas como indicadoras alternativas de contaminação fecal (SHAHRYARI *et al.*, 2015). A classe Gammaproteobacteria abriga várias espécies bacterianas de origem fecal, incluindo indicadoras de qualidade de água, como coliformes fecais (YE; ZHANG, 2012) e Flavobacteriia e Actinobacteria são táxons importantes na decomposição da matéria orgânica (LEWIN *et al.*, 2016). Alphaproteobacteria, Betaproteobacteria e Cytophagia foram positivamente relacionadas com fontes hídricas superficiais, apresentando parâmetros físico-químicos adequados para qualidade de água potável, como pH e potencial de oxirredução. De fato, microrganismos destas classes são frequentemente relatados em águas não poluídas (MLEJNKOVA; SOVOVA, 2010).

Interessantemente, as classes South African Gold Mine (arqueia), Nitrospira e Acidobacteria foram associadas positivamente com arsênio e nitrato e negativamente com pH e oxigênio dissolvido, condições detectadas em águas subterrâneas na estação chuvosa. De fato, bactérias representantes de Acidobacteria são encontradas, principalmente, em ambientes com pH baixo. South African Gold Mine é reconhecido como um grupo abundante de organismos que contribuem para a oxidação da amônia na maioria dos *habitats* terrestres e aquáticos com pH baixo (LEHTOVIRTA-MORLEY, 2016). Nitrospira, geralmente, é encontrada em ambientes aquáticos e participa do ciclo do nitrogênio, realizando a oxidação de nitrito (LUCKER *et al.*, 2010). Estas bactérias, frequentemente, ocorrem em íntima associação para converter amônia em nitrito e nitrito em nitrato (DAEBELER *et al.*, 2014), portanto, envolvidas em vários estágios do metabolismo de nitrogênio, sugerindo que este elemento pode ter um papel importante na cadeia trófica das águas subterrâneas avaliadas (TETU *et al.*, 2013).

Múltiplas associações também foram observadas entre OTUs e metais pesados. Destacam-se *Bacillus*, *Acinetobacter*, *Ralstonia*, *Staphylococcus*, *Burkholderia*, *Enterobacter*, *Sediminibacterium*, *Flavobacterium* e *Nistrospira*, que foram associados com mais de um metal e que, de fato, são frequentemente reportados pela sua resistência a metais pesados (OREMLAND *et al.*, 2004; RAJBANSHI, 2008; ABBAS *et al.*, 2014). Observou-se correlação entre o gênero *Enterococcus* e três metais (níquel, manganês e arsênio), o que pode estar

relacionado com sua frequente multirresistência antimicrobiana (TYNE; GILMORE, 2014). Alguns estudos revelam associações frequentes entre tolerância a metais pesados e resistência a fármacos antimicrobianos (PAL *et al.*, 2017).

Além dos clássicos patógenos entéricos frequentemente relacionados com contaminação fecal, há diversos outros patógenos ambientais, muitas vezes oportunistas, que podem colonizar as fontes de água para consumo humano (ASHBOLT, 2015a). Sequências que correspondem a bactérias potencialmente patogênicas foram detectadas tanto nas águas subterrâneas como nas águas superficiais, mas, no geral, detectadas com abundância inferior a 1%, como *A. baumannii*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Aeromonas caviae*, *Aeromonas simiae*, *Aeromonas veronii*, *Klebsiella oxytoca*, *Mycobacterium* sp., *Methylobacterium* sp., *Staphylococcus* sp., *Serratia* sp., *Moraxella* sp., *Legionella* sp*., Arcobacter* sp., *E. faecium*, *E. faecalis*, *Enterobacter* sp. e *E. coli*.

A fonte de água superficial “Buraco quente”, na estação chuvosa e seca, teve o padrão de abundância relativa aumentado dos gêneros *Arcobacter*, *Aeromonas*, *Pseudomonas*, *Klebsiella*, *Streptococcus*, *Enterobacter*, *Legionella*, *Clostridium*, *Moraxella*, *Salmonella*, *Bacillus*, *Escherichia-Shigella*, *Prevotella*, *Blautia* e *Enterococcus.* Interessantemente, essa fonte é usada de forma alternativa para consumo humano, cuja água não recebe tratamento adequado e, assim, os dados comprovam o risco potencial representado por ela. Em contraste, na fonte de água superficial “Novo Horizonte”, também utilizada de forma alternativa, sequências de bactérias potencialmente patogênicas foram ausentes ou foram detectadas em menores abundâncias, sugerindo uma água para consumo com qualidade microbiológica superior.

A outra abordagem deste estudo foi a análise da diversidade procariótica do sistema de tratamento de água potável principal de Ouro Preto (ETA Itacolomi). A metagenômica, buscando o sequenciamento de alto rendimento do rDNA 16S, tem contribuído para a caracterização de comunidades microbianas de sistemas de tratamento de água de diversos países que utilizam rios e lagos como fontes de água bruta para captação (LU *et al.*, 2016; VAZ-MOREIRA *et al.*, 2017). Entretanto, outras fontes de captação permanecem pouco estudadas. O sistema de tratamento de água do Itacolomi utiliza a captação de uma água bruta que nasce no alto do

Parque Estadual do Itacolomi e escorre sobre as pedras até ser captada e tratada por meio de vários processos, incluindo coagulação, floculação, decantação, filtração e desinfecção (adição de hipoclorito de sódio), anteriormente à sua distribuição.

O filo Proteobacteria, classe Betaproteobacteria, predominou nas amostras de água bruta, sendo consistente com dados frequentemente apresentados para outras águas brutas, como rios e lagos, que também são ambientes aquáticos dulcícolas (PINTO *et al.*, 2012; HUANG *et al.*, 2014). Em contrapartida, Firmicutes, classe Bacilli, dominou nas amostras de água tratada, ou seja, nas amostras pós-tratamento e nas amostras do ponto final de distribuição, mesmo na presença de cloro residual. Este resultado indica que o tratamento de água favoreceu a predominância de Firmicutes no sistema de tratamento de água. Até hoje, a importância de diferentes grupos, que não Proteobacteria, tem sido pouco relatada na resistência ao tratamento e persistência no sistema de distribuição. As OTUs de Firmicutes encontradas foram, principalmente, *Geobacillus* e *Bacillaceae* não classificada na estação chuvosa e *Enterococcus*, Bacillales não classificada e *Paenibacillus* na estação seca, que também foram detectadas nas amostras de água bruta, entretanto, em menor abundância. Alguns desses táxons são produtores de esporos e estão mais bem adaptados às condições ambientais que não favorecem a multiplicação microbiana, como exemplo, calor, solventes químicos, oxidantes, radiação ultravioleta e fungicidas (ABECASIS *et al.*, 2013). Assim, a liberação de esporos pode conferir resistência a estas bactérias, possibilitando a manutenção das mesmas nas águas pós-tratadas e cloradas. Entretanto, o gênero *Enterococcus* não é formador de esporos. Uma hipótese para sua persistência e dominância é sua resistência intrínseca a fármacos antimicrobianos. Estudos recentes mostram que a desinfecção da água impõe estresse à comunidade microbiana, o que pode estar associado a um aumento na prevalência e enriquecimento de bactérias resistentes a antimicrobianos em águas tratadas (SHI *et al.*, 2013; BAI *et al.*, 2015).

O estudo também mostrou, baseado nos índices de alfa-diversidade, que a comunidade procariótica da água bruta é mais diversa do que as amostras de água tratada e apresenta contagem superior, com base na quantificação do rDNA 16S. Em adição, a avaliação da beta-diversidade demonstrou perfil da comunidade

procariótica da água bruta distante das amostras de água tratada. Este resultado corrobora a literatura, que demonstra que o tratamento de água causa um efeito sobre a comunidade procariótica, alterando a diversidade e o perfil da composição taxonômica (GOMEZ-ALVAREZ *et al.*, 2012; CHAO *et al.*,2013).

Gêneros que incluem espécies patogênicas, como *Mycobacterium*, *Acinetobacter*, *Pseudomonas*, *Methylobacterium*, *Legionella*, *Arcobacter*, *Aeromonas, Enterococcus* e *Escherichia-Shigella*, foram detectados em amostras de água bruta, tratada e do ponto final de distribuição. De acordo com o banco de dados PATRIC, eles foram correlacionados intimamente com as sequências de *Mycobacterium intracellulare*, *A. baumannii*, *P. aeruginosa*, *Methylobacterium sp*., *Legionella* sp., *Arcobacter* sp., *A. hydrophila*, *E. coli* e *E. faecalis*. Neste estudo, particularmente *A. baumannii*, *E. faecalis* e *Methylobacterium* sp. aumentaram sua abundância relativa até o ponto final de distribuição. Este resultado sugere a resistência dessas bactérias ao tratamento de água potável e persistência mesmo na presença de cloro residual no sistema de distribuição.

De fato, sabe-se que a cloração pode inibir certos microrganismos, enquanto seleciona outros, dentre eles patógenos oportunistas que são relativamente resistentes ao cloro, como *Mycobacterium*, *Acinetobacter* e *Methylobacterium* (INGERSON-MAHAR; REID, 2012; LIU *et al.*, 2016). A detecção de bactérias potencialmente patogênicas ao longo do sistema de tratamento de água pode ser alarmante, principalmente, por aumentar os riscos de doenças relacionadas à água e problemas de saúde, particularmente, em indivíduos imunocomprometidos (LIU *et al.*, 2016). Essas bactérias são de particular importância para a saúde pública em ambientes hospitalares, pois são agentes de infecções relacionadas à assistência à saúde (IRAS). Entretanto, embora a presença de sequências gênicas destas amostras não confirme sua viabilidade, sua presença é um sinal de preocupação para a saúde pública.

6 SÍNTESE DOS RESULTADOS E CONCLUSÃO

Em síntese,

- O filo Proteobacteria, a classe Betaproteobacteria e a OTU *Comamonadaceae* não classificada foram os táxons dominantes na maioria das fontes de água doce para consumo. Como exceção, nas águas subterrâneas na estação seca, o filo Firmicutes, a classe Bacilli e os gêneros *Enterococcus* e *Bacillus* dominaram.

- Os índices de alfa-diversidade das fontes superficiais foram semelhantes e não mostraram alteração nas diferentes estações climáticas estudadas. A beta-diversidade também demonstrou similaridade quanto ao perfil das comunidades procarióticas entre as fontes superficiais. Contudo, as fontes de água subterrânea mostraram ser influenciadas pelo clima, pois, na estação chuvosa os índices de diversidade e riqueza aumentaram significativamente e o perfil taxonômico foi diferente.

- As características físico-químicas variaram significativamente entre as comunidades procarióticas das fontes de água subterrânea e superficial. As classes Bacteroidia, Flavobacteria, Gammaproteobacteria, Clostridia e Actinobacteria foram relacionadas com parâmetros que indicam poluição orgânica. Em contrapartida, Alphaproteobacteria, Betaproteobacteria e Cytophagia foram associadas com parâmetros característicos de água com qualidade adequada. South African Gold Mine, Nitrospira e Acidobacteria foram associadas com presença de nitrato e arsênio e valores de pH e OD baixos. A classe Bacilli foi relacionada apenas com pH e OD baixos.

- Alguns gêneros foram correlacionados com níquel, ferro, arsênio e manganês detectados nas fontes de águas, evidenciando a resistência de várias bactérias a metais pesados.

- Espécies de bactérias potencialmente patogênicas relevantes foram detectadas nas fontes de água superficial e subterrânea. O padrão de

abundância relativa dessas bactérias foi maior em algumas fontes, revelando o risco potencial dessas águas. Em contrapartida, algumas águas mostraram um padrão de abundância dessas bactérias menor, sendo, assim, consideradas fontes de água mais preservadas.

- A estrutura procariótica em nível taxonômico de filo e classe foi alterada pelo tratamento da água; enquanto o filo Proteobacteria, classe Betaproteobacteria, predominou na água bruta e o filo Firmicutes, classe Bacilli, foi o mais comumente observado na água pós-tratada e no ponto final de distribuição.
- Os índices de alfa-diversidade e o número de cópias do rDNA 16S bacteriano foram maiores em amostras brutas do que em amostras tratadas (pós-tratamento e ponto final de distribuição). A beta-diversidade demonstrou também diferenças do perfil da comunidade procariótica das amostras de água bruta em comparação com as amostras de água tratada.
- Foram detectadas bactérias potencialmente patogênicas, como *E. coli*, *E. faecalis*, *Mycobacterium intracellulare*, *A. baumannii* e *P. aeruginosa*, indicando a resistência dessas bactérias ao tratamento e persistência das mesmas na água para consumo humano.

Em conclusão, nosso trabalho demonstrou que o sequenciamento de alto rendimento do rDNA 16S pode fornecer uma visão abrangente da estrutura e diversidade das comunidades procarióticas e do padrão de distribuição de grupos potencialmente patogênicos nas águas utilizadas para consumo humano. Destaca-se, neste estudo, o filo Firmicutes, classicamente pouco abundante em água doce, que dominou em águas subterrâneas e demonstrou versatilidade ao persistir a todas as etapas de tratamento do sistema de tratamento de água estudado. Além disso, é importante destacar que a água municipal de Ouro Preto exibe evidências de contaminação bacteriana substancial e que os microrganismos estão sendo continuamente transmitidos através do sistema de abastecimento de água, dentre eles, bactérias potencialmente patogênicas. No futuro, este tipo de informação pode subsidiar o estabelecimento de estratégias de tratamento mais eficazes, adaptadas

à diversidade única presente em cada fonte e sistema de tratamento de água para consumo humano.

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