



Review article

A systematic scoping review of antibiotic-resistance in drinking tap water



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ABSTRACT

Environmental matrices have been considered of paramount importance in the spread of antibiotic-resistance; however, the role of drinking waters is still underexplored. Therefore, a scoping review was performed using a systematic approach based on PRISMA guidelines, with the aim of identifying and characterizing antibiotic-resistance in tap water, specifically, water treated at a potabilization plant and provided for drinking use through a water distribution system. The review included 45 studies, the majority of which were conducted in upper-middle-income economies (42.2%), mainly from the Western Pacific region (26.7%), followed by Europe (24.4%). Most of the papers focused on detecting antibiotic-resistant bacteria (ARB), either alone (37.8%) or in combination with antibiotic-resistant genes (ARGs) (26.7%). Multidrug-resistance profile was often identified in heterotrophic bacteria, including various species of nontuberculous mycobacteria, *Pseudomonas* spp., and *Aeromonas* spp., which were especially resistant to penicillins, cephalosporins (including 3rd-generation), and also to macrolides (erythromycin) and tetracyclines. Resistance to a wide range of antibiotics was also prevalent in fecal bacteria, e.g., the *Enterobacteriaceae* family, with common resistance to (fluoro)quinolones and sulfonamide groups. ARGs were investigated either in bacterial strains isolated from tap waters or directly in water samples, and the most frequently detected ARGs belonged to β -lactam, sulfonamide, and tetracycline types. Additionally, mobile genetic elements were found (i.e., *int1* and *tnpA*). Sulfonamides and macrolides were the most frequently detected antibiotics across countries, although their concentrations were generally low (<10 ng/L) in Europe and the United States. From a health perspective, tap water hosted ARB of health concern based on the 2024 WHO bacterial priority pathogens list, mainly *Enterobacteriaceae* resistant to 3rd-generation cephalosporin and/or carbapenem. Despite the fact that tap water is treated to meet chemical and microbiological quality standards, current evidence suggests that it can harbor antibiotic-resistance determinants, thus supporting its potential role in environmental pathways contributing to antibiotic resistance.

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1. Introduction

Antimicrobial resistance (AMR) is a global public health threat and one of the greatest worries about AMR is represented by antibiotic resistance phenomenon, because antibiotics are widely used for clinical and prophylactic treatments in human health systems, but veterinary medicine also relies heavily on antibiotics (O'Neill, 2014). Antibiotic-resistance has been recently considered a *quintessential One Health issue*, given the interconnection among human, animal and ecosystem domains in the antibiotic-resistance spread (WHO, 2022a) and the awareness of the environmental role in such triad is progressively increasing (UNEP, 2022, 2023). It is well demonstrated that antibiotic-resistance determinants may not be entirely removed from wastewater treatment plants (WWTPs), and they can behave as hotspot sources of antibiotic-resistance bacteria (ARB), antibiotic resistance genes (ARGs) (Rizzo et al., 2013; Gwenzi et al., 2020; Bonetta et al., 2023), and also antibiotics (Sanseverino et al., 2018). In turn, surface water as well as groundwater receiving sublethal concentrations of antibiotics (in the order of ng/L; Sanseverino et al., 2018) can promote the selection of ARB and ARGs. Once in the drinking water supplies, antibiotic-resistant determinants can pass through drinking water treatment plants (DWTPs), because some treatments show very little ARG abatement (and even promotion effect of ARGs), as in the case of sand filtration toward chloramphenicol resistant genes and activated carbon filtration toward ARGs providing resistance to sulfonamide and quinolone (Zheng et al., 2018; Su et al., 2018). Moreover, chlorination, frequently used as final disinfection stage for potable water production, could enhance dissemination of antibiotic resistance by increasing total relative abundance of various ARGs (Guo et al., 2014; Jia et al., 2015). Such effect mainly happens at low concentration of chlorine, that could exert co-selection mechanisms and improve horizontal gene transfer (HGT) as a result of cell membranes permeabilization that increases both transformation (acquisition of extracellular ARGs) and conjugation (ARG exchange among different bacteria genera) phenomena (Sanganyado and Gwenzi, 2019; Gao and Sui, 2021). These literature data are also confirmed by a scientific report to the Water Research Commission, that addressed the monitoring of waters at the inlet and at the outlet of various DWTPs with different treatment schemes; it showed that various bacterial species isolated from the raw waters and the finished drinking waters had similar antibiotic resistance and virulence phenotypes (Bezuidenhout et al., 2019).

Therefore, populations can be exposed to antibiotic-resistant determinants through the ingestion of household waters. In fact, in many countries throughout the world, the household waters are suitable for human consumption and people cover most of their daily water requirement by drinking water directly from the tap (ECORYS, 2015). Health effects of antibiotic-resistance in drinking waters are still largely unexplored, although WHO suggested three possible adverse outcomes (WHO, 2015). The first is human infection by ARB, as confirmed by some outbreaks where the integration between epidemiological surveillance data and environmental monitoring of drinking waters confirmed the role of such a matrix as the vehicle of the resistant pathogens, e.g., *Shigella sonnei* resistant to azithromycin and 3rd generation cephalosporin in China (Ma et al., 2017) and multidrug resistant *Salmonella typhi* in Nepal (Lewis et al., 2005). The second mechanism is the gut colonization by resistant microbes (e.g., *Escherichia coli*), that is supported by an epidemiological evidence from Coleman et al. (2012), who performed a cross-sectional study showing an association between drinking water consumption and the presence of β -lactam resistant *E. coli* in human feces (namely resistance to,

e.g., penicillin and cephalosporins). The third mechanism is ARG transfer to normal microflora, according to a hypothesis early suggested by Salyers et al. (2004) who highlighted the increase of tetracycline- and erythromycin-resistant *Bacteroides* spp. in human stools comparing community colon isolates before the use of antibiotics in human medicine and during the antibiotic era. Such hypothesis has been recently demonstrated through *in vitro* and *in vivo* experiments. In particular, Zhou et al. (2022) found that extracellular ARGs showed high gene horizontal transfer potential passing through an artificial digestive tract, especially ARGs against tetracyclines; similar results were obtained by Khan et al. (2020) who spiked the feeding waters of mice with colistin-resistant *Bacillus cereus* and found that such resistance had been transferred to *Enterococcus hirae*, that is an intestinal indigenous bacterium. Therefore, antibiotic-resistant bacteria and genes in tap waters can pose potential risks to human health. Although to date, there is no legislative requirement for testing drinking waters for antibiotic-resistant determinants, the scientific interest on this topic is rapidly increasing. Thus, this work was aimed at collecting the available evidences on ARB, ARGs, and antibiotics in drinking tap waters.

2. Material and methods

2.1. Review type and research team

The scientific literature has been investigated through a scoping review (ScR; Peters et al., 2020), which is suited to identify and describe relevant evidence on an existing or emerging topic using a broader research question, as described by the Joanna Briggs Institute (e.g., Peters et al., 2015, 2020; Munn et al., 2018). To increase methodological transparency and uptake of research findings, the ScR was conducted following the Preferred Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, adapted for ScR by Tricco et al. (2018) and already applied for knowledge synthesis of environmental science topics (Corrin et al., 2024).

Prior to conducting the ScR, a multidisciplinary team (represented by the authors of the present paper) with expertise in environmental hygiene, public health, microbiology, and evidence synthesis discussed and approved a protocol that included the following information: research question, literature search strategy (search string and database), inclusion/exclusion criteria, and data charting form for the extraction of the information from the papers, as detailed below.

2.2. Research question and eligibility criteria

The goal of the ScR was to investigate the current evidence that address the research question: Which ARB, ARGs or antibiotics have been detected in drinking tap waters? The inclusion criteria were.

- (1) Publication date: no time limitation
- (2) Language: literature published in English
- (3) Document type: Primary research, namely monitoring studies where the authors collected and analyzed their own data
- (4) Type of waters: waters that received potable treatment and that were distributed to the communities via drinking water distribution systems.

Moreover, we considered methodological rigor as a further eligibility criterion, based on a Kmet checklist (Kmet et al., 2004) for evaluating primary research papers from various fields. In particular, we considered eligible for the inclusion in the present ScR, the papers that reported the question/objective sufficiently described, the study design evident and appropriate, subject characteristics sufficiently described, and sample size appropriate (> or = 10 tap water samples analyzed, to provide

adequate representativeness of such matrix), analytical methods described/justified and appropriate, and results reported in sufficient details.

Exclusion criteria are reported below.

- Articles not published in English
- Reviews and non-primary literature (e.g., commentaries, opinions, letter to the editors)
- Articles on untreated or partially treated waters within a DWTPs (e.g., water supplies, either fresh or ground waters, water at the exit of each treatment step) or treated but not piped into a distribution system (e.g., water immediately at the exit of final disinfection)
- Articles on waters used for human consumption but that either i) did not receive any treatment (e.g., well or bottled waters), or ii) it was not clear whether they had received treatment or not
- Articles reporting aggregated data, namely data on tap waters were presented as a whole with results on other analyzed environmental matrices (e.g., raw water supplies, surface waters, spring waters, sewages, water at different stages of the potabilization treatment) thus hampering the extraction of data specific to tap waters
- Articles whose methodology was not compliant with the criteria selected from Kmet et al. (2004), namely small sample size (<10 samples), analytical methods not adequately described, such as the lack of limit of detection (LOD) and limit of quantification (LOQ) for papers on antibiotics, and results not clearly explained (e.g., information needed to be inferred from figures).

2.3. Literature search strategy and study selection

The search string includes the following terms (“antibiotic-resistant bacteria” OR “antibiotic-resistant gene” OR ARB OR ARG OR antibiotic) AND (“tap water” OR “potable water” OR “finished water” OR “drinking water”). Searches were conducted on February 6th, 2023, using three electronic databases: PubMed (search field = all fields), Scopus (search field = article title, abstract, and keywords), Web of Science (WoS) core collection (search field = topic). Searches were then updated on August 28th, 2024 using identical search string. The records were cleaned of duplicates using the Zotero platform (Corporation for Digital Scholarship) (Fernandez, 2011). The study selection was performed in two stages (e.g., Boehm et al., 2018). The initial screening for eligibility entailed reading of the title and abstract. It was purposely inclusive, therefore if the abstract was not available and/or the relevance of the article could not be determined from the title, the document was retrieved for full reading. Each article that passed initial screening was subjected to full-text screening. The two-step study selection was done by five reviewers. At each stage, a selection of 20 articles was reviewed by all the above-mentioned investigators to reach consensus about applying the eligibility criteria. After that, each investigator independently assessed the retrieved articles (Lenzen et al., 2017). Any doubt raised during the screening process was resolved through periodic online meetings among the entire research team.

2.4. Data collection and management

A data-charting form was jointly developed by the research team to determine which variables to extract from the articles that met the eligibility criteria. In particular, the following variables were considered: year of publication, country, sample size, sample volume, targets of the monitoring (separately for ARB, ARGs, and antibiotics), analytical methods, and results (types and occurrence of ARB, ARGs, or antibiotics). The data charting process was performed independently by the above-mentioned reviewers, who periodically discussed their results and continuously updated the data-charting form.

The results were globally presented in terms of geographical area based on WHO region (WHO, 2024a) and the United Nations Statistics Division (UNSD, 2024), income level (World Bank, 2024), and temporal distribution of the included articles. Then, information on methodological approaches and main findings were synthesized separately for ARB, ARGs, and antibiotics. For the purpose of the present study, ARB were divided into fecal and environmental types, based on the matrix where they are mostly detected. Thus, fecal ARB included bacteria from *Enterobacteriaceae* family and *Enterococcus* genus, and the other types of microbes, including heterotrophic plate count (HPC) flora, were considered environmental ARB. Moreover, the occurrence of ARB of particular health concerns was also considered and discussed, given the relevance of tap waters for public health. For the classification of these types of ARB, the recent update of the WHO Bacterial Priority Pathogens List (BPPL) was considered (WHO, 2024b), that spans different families of antibiotic-resistant bacterial pathogens. This list classifies the ARB into 3 priority groups depending on the need of research and development of new antibiotics, given their global impact in terms of burden and issues related to transmissibility, treatability, and prevention options. The scheme of WHO BPPL used for classifying the health-relevance of ARB detected in tap waters is reported in Table S1.

3. Results and discussion

3.1. Search results and overall study characteristics

The search of the published literature yielded 11,993 articles: 3277 from Pubmed; 4623 from Scopus; 4093 from WoS (Fig. 1). A total of 152 papers were selected for retrieval on the basis of the inclusion criteria. Of these, 107 were excluded after full reading for the following reasons: 21 articles not focused on drinking tap water (e.g., water at various stages of drinking-water supply chain, well waters, bottle waters, tap waters not used for drinking purposes); 9 articles did not make it clear whether the tap water was treated or not; 18 articles reporting aggregated data; 41 articles provided a methodology that was not considered eligible for the present ScR, mainly given the small sample size. Moreover, 18 papers were also excluded because they were not relevant for the ScR, since they showed different goals related to tap waters (e.g., investigating the role of biofilm, studying the antibiotic-resistance determinants in microcosms) (“Other” category in Fig. 1). The studies that did not meet the criteria are listed in Table S2 with the reasons of exclusion. Overall, 45 articles were included in the review and considered eligible for the assessment, the main characteristics of which are summarized in Table S3.

The first paper appeared in 1988, and the publishing rate showed a gradual increase starting from 2005. Then, since 2017, the number of papers has markedly increased (Fig. 2), probably as a result of the global concerns and awareness about antimicrobial resistance, as shown by internationally relevant documents, such as the 2016 United Nations political declaration on AMR (UN, 2016) and the first BPPL released by WHO in 2017 on ARB for which the development of new antibiotics is urgently needed (WHO, 2017). The geographical distribution indicates the predominance of the papers carried out in Asian countries, in fact, in terms of UNSD regions, the most representative were Eastern Asia (24.4%), followed by Southern Asia (13.3%) and Western Asia and Eastern Europe (8.9%, each). In fact, considering WHO regions, the most frequently represented areas were Western Pacific region (26.7%) and Europe (24.4%). The most represented countries were China (10/45, 22.2%) and Poland (4/45, 8.9%). Most of the studies were performed in upper-middle-income economies (42.2%) followed by high-income and lower-middle countries (28.9%, each), but none were conducted in low-income economies (Table S4; Fig. 3). Regarding the aim of the

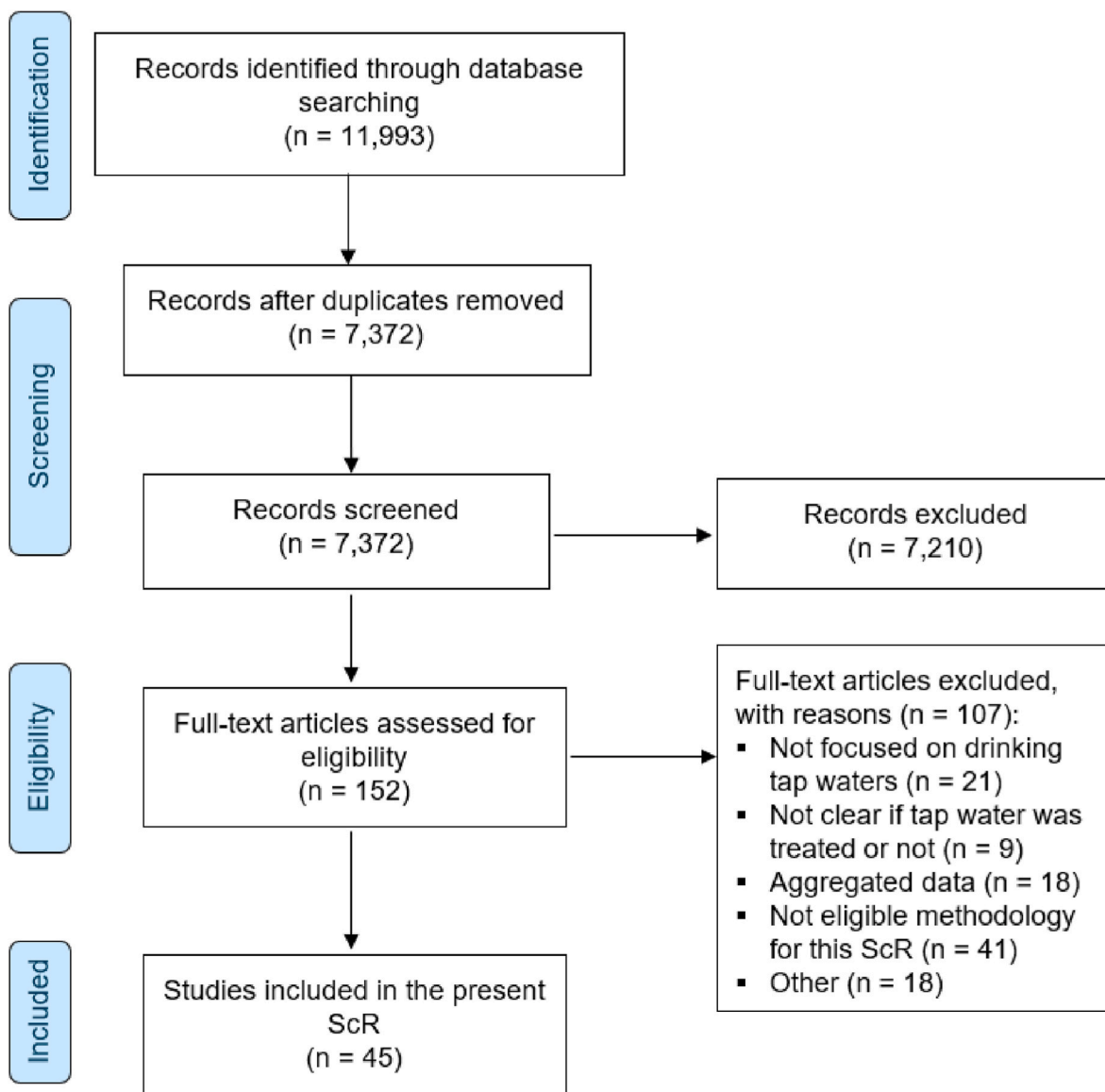


Fig. 1. PRISMA flow diagram of the articles of the scoping review process on antibiotic-resistance in tap waters.

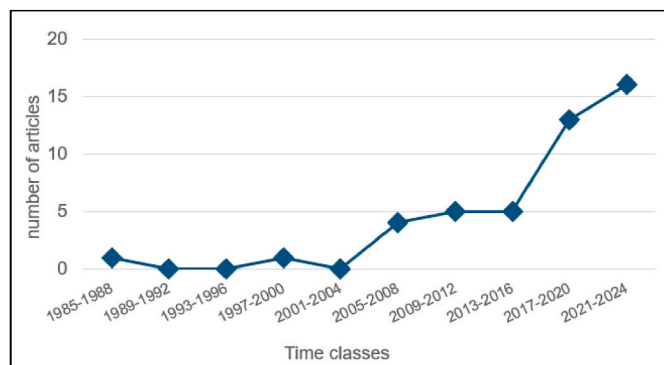


Fig. 2. Time-trend of the included articles (4-year classes). The first article has been published in 1988. For 2024, the search is limited to eight months.

monitoring, in most cases, the studies were aimed at investigating ARB alone (17/45, 37.8%) or in combination with ARG (12/45, 26.7%), while the rest of the papers were focused only on the monitoring of ARGs (10/45, 22.2%) and antibiotics (6/45, 13.3%). Therefore, the investigation of tap waters for antibiotic-resistance is performed preferentially using culture-based methods for establishing phenotypic profiles of ARB. Such a result differs from data reported by [Siri et al. \(2023\)](#) in a water environment that showed a widespread use of molecular methods.

3.1.1. Tap water definition

Given the heterogeneous origin of the selected papers, water quality at the point of use needs to be further explored since tap water requirements can vary in different countries. At the international level, the reference for the establishment of national/regional regulations for water safety is represented by the guidelines for drinking-water quality (GDWQ), released by the WHO since 1958 and recently updated ([WHO, 2022b](#)). The verification and surveillance of microbial water quality are

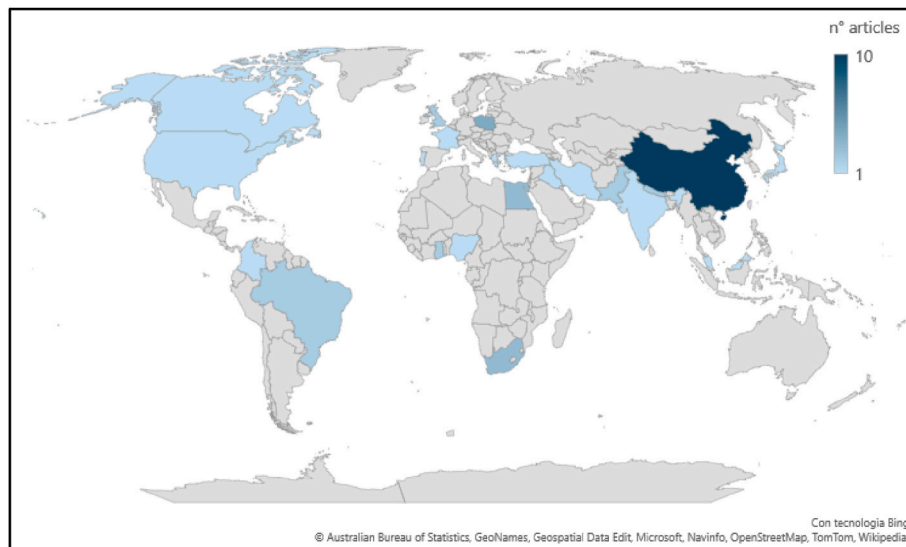


Fig. 3. Geographical distribution of the included articles (the darkest the color the highest the number of the articles published by the country).

based on the monitoring of fecal indicators, namely *E. coli* or thermo-tolerant coliforms, that should be absent per volume in all waters intended for human consumption as well as treated water at the entrance and at the exit of the distribution system (guidelines value 0/100 ml; WHO, 2022b). Nevertheless, GDWQ represents guidance for the development of countries' own regulations, therefore they can be adapted to local conditions, circumstances, needs, and resources of countries (WHO, 2021). As an example, some countries fail to meet the requirement for microbial water safety and allow that coliform bacteria may be detected in samples on occasions, also considering that drinking-water of a particular quality may lead to different health effects in different populations, given the variable susceptibility to pathogens (WHO, 2022b). In developed countries, the loss of compliance occurs occasionally and can be the result of, e.g., deterioration in source water quality, failures associated with treatment processes or the integrity of distribution systems, and inadequate disinfection (CDC-EPA-AWWA, 2016; Galway, 2016). In low and middle-income countries, deterioration of tap water is frequent, especially owing to the lack of adequate supply infrastructure for water distribution. In these areas, even if the water is treated adequately by the potabilization facility, low and intermittent water pressure within the piped water supply system is common as a result of water shortage and rupture of the distribution networks, thus drawing the surrounding contaminants into the water supply (Mermin et al., 1999; Shakya et al., 2022), but also cross-contamination of drinking water with sewage (Qamar et al., 2018; Lewis et al., 2005). When tap water exceeds microbial water quality standards, point-of-use household water treatments (e.g., boiling) are recommended by water suppliers and public health authorities before drinking, thus reducing the exposure to pathogens via ingestion (WHO, 2015; WHO, 2022b). Nevertheless, unsafe tap water can be used for other household purposes, e.g., showering, washing clothes, toilet flushing, which could lead to exposure to microbes via accidental swallowing, inhalation or contact with intact skin or wounds.

3.2. Antibiotic resistant bacteria

Most of the reviewed articles (29/45) were focused on the investigation of ARB, of which fourteen investigated environmental ARB (Ateba et al., 2020; Dávalos et al., 2021; Emekdas et al., 2006; Ezzat, 2014; Furuhashi et al., 2006; Leginowicz et al., 2018; Moghaddam et al., 2022; Molale-Tom et al., 2024; Siedlecka et al., 2020, 2021; Scoaris et al., 2008; Vaz-Moreira et al., 2012; Walsh et al., 2011; Zhang et al., 2018), ten monitored fecal ARB and staphylococci (Adzitey et al., 2016; Akbar et al., 2022; Bhatta et al., 2007; Elmi et al., 2021; Elmonir et al., 2020; Hamza et al., 2020; Kinge et al., 2010; Papandreou et al., 2000; Santos et al., 2023; Subba et al., 2013) and five articles investigated both environmental and fecal ones (Ahmed et al., 2022; Adesoji et al., 2017; Borjac et al., 2023; Jazrawi et al., 1988; Siedlecka and Piekarska, 2019). The characteristics of such articles are summarized in Table 1 and described below.

The presence of ARB was evaluated in less than 50 samples in more than half of the reviewed articles (16/29) (Table S3). Sample analysis and bacterial isolation methods relied mostly on the membrane filtration technique, which represents the standard procedure for bacteriological examination of drinking waters. Briefly, water samples are filtrated on a 0.45 μm pore size membrane filter, then the membrane is incubated on a selective agar plate, then single colonies are selected for subsequent analysis of identification and antibiotic susceptibility testing. In some cases, authors introduced modifications to the procedure, such as the pore size (0.22 μm) or the usage of agar media already supplemented with an antibiotic. Only few studies applied different sample analysis approaches, such enrichment culture method following by culturing onto selective media and bacterial precipitation by centrifugation (Table 1).

The identification of the isolates occurred mainly via biochemical techniques, frequently followed by genotypic identification of the selected isolates by sequencing the 16S rRNA gene. In the case of HPC, the identity of the isolates was determined using only a genotypic approach (Table 1).

The preferred method for evaluating antibacterial activity was the

Table 1
Methodological aspects and types of antibiotic-resistant bacteria detected in the reviewed articles on tap waters.

Authors (country)	Category of bacteria investigated	Sample concentration and bacterial isolation	Methods for bacterial identification	Analytical method for verifying sensitivity to antibiotics	Types of ARB families or genus detected
Adesoji et al., 2017 (Nigeria)	Environmental, fecal	No concentration method. 1-ml samples were serially diluted and cultured on nutrient or selective agar media, depending on the searched microbes	Genotypic techniques	Agar dilution method	Various psychro- and mesophilic bacteria species; various species of <i>Enterobacteriaceae</i> family
Adzitey et al., 2016 (Ghana)	Fecal	Enrichment culture method	Biochemical techniques	Disc diffusion	Salmonella spp.
Ahmed et al., 2022 (Ghana)	Environmental, fecal	Membrane filtration technique	MALDI-TOF MS	Disc diffusion	<i>Pseudomonas</i> spp.; various species of <i>Enterobacteriaceae</i> family
Akbar et al., 2022 (Pakistan)	Fecal	Enrichment culture method	Biochemical and genotypic identification techniques	Disc diffusion	<i>Escherichia coli</i>
Ateba et al., 2020 (South Africa)	Environmental	Membrane filtration technique	Genotypic technique	Disc diffusion	HPC bacteria
Bhatta et al., 2007 (Nepal)	Fecal	Membrane filtration technique	Biochemical techniques	Disc diffusion	Various Salmonella species
Borjac et al., 2023 (Lebanon)	Environmental, fecal, other	- For psychrophilic bacteria: No concentration method. 1-ml samples were serially diluted and cultured on nutrient agar media (R2A agar) - For other target: Membrane filtration technique	MALDI-TOF MS	Disc diffusion	Various species of psychrophilic bacteria; <i>Pseudomonas aeruginosa</i> ; <i>Staphylococcus</i> spp.; <i>E. coli</i> ; <i>Enterobacteriaceae</i> family
Dávalos et al., 2021 (Colombia)	Environmental	Membrane filtration technique with some modifications	Biochemical techniques followed by genotypic identification	Broth dilution method	Nontuberculous mycobacteria
Elmi et al., 2021 (Malaysia)	Fecal	Enrichment culture method	Biochemical techniques	Disc diffusion	<i>Escherichia coli</i>
Elmonir et al., 2020 (Egypt)	Fecal	Multiple fermentation tube technique	Biochemical techniques	Disc diffusion	<i>Escherichia coli</i>
Emekdas et al., 2006 (Turkey)	Environmental	Enrichment culture method	Biochemical techniques followed by genotypic identification	Disc diffusion	<i>Aeromonas</i> spp.
Ezzat 2014 (Egypt)	Environmental	Membrane filtration technique	Biochemical techniques	Disc diffusion	<i>Aeromonas</i> spp.
Furuhata et al., 2006 (Japan)	Environmental	No concentration method. Samples were cultured on nutrient agar media (R2A agar)	Biochemical techniques followed by genotypic identification	Disc diffusion	<i>Methylobacterium</i> spp.
Hamza et al., 2020 (Egypt)	Fecal	Membrane filtration technique followed by enrichment method	Biochemical techniques	Disc diffusion	Various species of <i>Enterobacteriaceae</i> family
Jazrawi et al., 1988 (Iraq)	Environmental, fecal	Membrane filtration technique	Biochemical techniques	Disc diffusion	<i>Pseudomonas</i> spp., various psychro- and mesophilic bacteria species; various species of <i>Enterobacteriaceae</i> family
Kinge et al., 2010 (South Africa)	Fecal	Membrane filtration technique	Biochemical techniques	Disc diffusion	<i>Escherichia coli</i>
Leginowicz et al., 2018 (Poland)	Environmental	Membrane filtration technique with some modifications (0.22 µm pore size filters)	Biochemical techniques followed by genotypic identification	Disc diffusion	<i>Pseudomonas</i> spp., various psychro- and mesophilic bacteria species
Moghaddam et al., 2022 (Iran)	Environmental	Membrane filtration technique	Biochemical techniques followed by genotypic identification	Broth dilution	Nontuberculous mycobacteria
Molale-Tom et al., 2024 (South Africa)	Environmental	No concentration method. 1-ml samples were serially diluted and cultured on nutrient agar media (R2A agar)	Genotypic technique	Disc diffusion	HPC bacteria
Papandreou et al., 2000 (Greece)	Fecal	Membrane filtration technique	Biochemical technique	Disc diffusion and broth dilution methods	Various species of <i>Enterobacteriaceae</i> family
Santos et al., 2023 (Brazil)	Other	Membrane filtration technique	Genotypic technique and MALDI-TOF MS for species identification	Disc diffusion	Coagulase-negative <i>Staphylococcus</i> spp.
Scoaris et al., 2008 (Brazil)	Environmental	Membrane filtration technique (0.45 µm)	Biochemical techniques followed by genotypic identification	Disk diffusion	<i>Aeromonas</i> spp.

(continued on next page)

Table 1 (continued)

Authors (country)	Category of bacteria investigated	Sample concentration and bacterial isolation	Methods for bacterial identification	Analytical method for verifying sensitivity to antibiotics	Types of ARB families or genus detected
Siedlecka and Piekarska 2019 (Poland)	Environmental, fecal	Membrane filtration technique with modifications (0.22 µm pore size filters; usage of agar supplemented with antibiotic – agar type varies according to the searched microbe)	Not specified	Agar dilution method	HPC bacteria; <i>Klebsiella pneumoniae</i> , <i>Enterococcus faecium</i> , <i>Enterococcus faecalis</i>
Siedlecka et al., 2020 (Poland)	Environmental	Membrane filtration technique with modifications (0.22 µm pore size filters; usage of nutrient agar media (R2A agar) supplemented with antibiotic)	Genotypic technique	Agar dilution method	HPC bacteria
Siedlecka et al., 2021 (Poland)	Environmental	Membrane filtration technique with modifications (0.22 µm pore size filters, usage of nutrient agar media (R2A agar) supplemented with antibiotic)	Biochemical techniques followed by genotypic identification	Agar dilution method	Various psychrophilic bacteria species
Subba et al., 2013 (Nepal)	Fecal	Membrane filtration technique	Biochemical techniques	Disk diffusion	<i>Escherichia coli</i>
Vaz-Moreira et al., 2012 (Portugal)	Environmental	Membrane filtration technique	Biochemical techniques followed by genotypic identification	Automatized system	<i>Pseudomonas</i> spp.
Walsh et al., 2011 (India)	Environmental	Precipitation by centrifugation	Genotypic technique	Broth dilution method	<i>Pseudomonas</i> spp., various psychro- and mesophilic bacteria species
Zhang et al., 2018 (China)	Environmental	No concentration method. 1-ml samples were serially diluted and cultured on nutrient agar medium supplemented with antibiotic	Genotypic technique	Agar dilution method	HPC bacteria

HPC = Heterotrophic Plate Count; MALDI-TOF-MS = matrix-assisted laser desorption ionization–time of flight mass spectrometry.

agar disk-diffusion method, which is a well-known procedure, where a filter paper disk containing a desired concentration of a certain antibiotic is placed on the surface of agar plates inoculated with the tested bacteria. In particular, this method has been applied by all the reviewed articles focusing on fecal ARB, while the analysis of antibiotic-resistance in environmental strains was performed also using the dilution method. The dilution method involves the incorporation of the desired antibiotic in a growth medium (agar or broth) containing the bacterial inoculum and allows the testing of several dilution of the desired antibiotic, thus allowing the determination of the Minimum Inhibitory Concentration (MIC) value. Overall, the authors distinguished the isolates in resistant or susceptible to a given antibiotic according to international committees (i.e., Clinical and Laboratory Standards Institute – CLSI, or the European Committee on Antimicrobial Susceptibility Testing - EUCAST), which provides guidelines for interpreting the MIC breakpoint or the inhibition zone diameter breakpoint. However, this approach is considered with caution by reviewed articles on environmental strains, as detailed in Sect. 3.2.1.

3.2.1. Antibiotic-resistance features in environmental ARB

Nontuberculous mycobacteria (NTM) (2/29 articles) were found resistant to different classes of antibiotics, depending on the NTM species. Moghaddam et al. (2022) found that more than half of the tested isolates of *M. aurum* were resistant to 2nd-generation cephalosporin (cefoxitin), *M. phocaicum* to fluoroquinolone class (ciprofloxacin), while *M. mucogenicum* and *M. fortuitum* showed major resistance to tetracyclines (doxycycline) and to carbapenems (meropenem and imipenem). Similar results were obtained by Dávalos et al. (2021), who detected NTM species also resistant to aminoglycosides (tobramycin).

Aeromonas spp. (3/29 articles) exhibited wide antibiotic-resistance patterns toward various compounds. Ezzat (2014) found all the tested isolates (more than 70 isolates) resistant to various types of penicillins (i.

e., amoxicillin-clavulanic acid, ampicillin, methicillin, and piperacillin), 1st generation cephalosporins (cephalothin), macrolides (erythromycin), glycopeptides (vancomycin) and lincosamides (clindamycin). These results were in accordance with those observed by Scoaris et al. (2008) and Emekdas et al. (2006), although Scoaris et al. (2008) also found isolates resistant to 3rd generation cephalosporins (cefotaxime) and chloramphenicols. Interestingly, in all three studies *Aeromonas* spp. isolates were susceptible to fluoroquinolones.

Pseudomonas spp. (6/29 articles) showed wide resistance (>50% of the tested isolates) to monobactams (aztreonam) (Ahmed et al., 2022), 3rd-generation cephalosporins (Borjac et al., 2023; Walsh et al., 2011), tetracyclines and epoxide (Vaz-Moreira et al., 2012). Resistance against penicillin classes varied according to the type of compound, with percentages of resistant isolates varying between 50% and 100% for ampicillin (Jazrawi et al., 1988; Leginowicz et al., 2018) and ticarcillin (Vaz Moreira et al., 2012) compared to piperacillin (resistance less than 10% of the tested isolates; Ahmed et al., 2022). Similarly, also resistance to fluoroquinolones depended on the type of molecule: 80% of the isolates resulted resistant to nalidixic acid (Vaz-Moreira et al., 2012) and only 5% to ciprofloxacin (Ahmed et al., 2022).

Other types of environmental bacteria have been also considered by seven of the reviewed articles that tested various isolates of psychro- and mesophilic aerobic bacteria. They were generally cultured and isolated on nutrient agar media, often R2A agar, because of its low-nutrient and low-ionic strength, then incubated at 22 °C or 37 °C for the investigation of psychrophilic or mesophilic bacteria, respectively. Overall, authors found multi-drug resistant (MDR) profiles (resistance to three or more antibiotics) in numerous groups of Gram-negative and Gram-positive bacteria (e.g., *Bacillus* spp., *Acinetobacter*, *Chromobacterium*, *Lysinibacillus*, *Psychrobacter*, *Brevundimonas*, *Myroides*). As an example, *Bacillus* spp. showed resistance in more than 50% of the tested isolates to 1st and 3rd generation cephalosporins and monobactam (Adesoji et al., 2017;

Leginowicz et al., 2018). *Acinetobacter johnsonii* showed resistance to 3rd (ceftazidime, ceftriaxone, cefotaxime) and 4th (cefepime) generation cephalosporins with percentages of resistant isolates ranging from 21% to 36% (Borjac et al., 2023). Some isolates of *Brevundimonas* spp. were resistant to aminoglycosides, penicillins and tetracyclines (Leginowicz et al., 2018) as well as fluoroquinolones and 3rd-generation cephalosporins (Siedlecka et al., 2021). *Methylobacterium* spp. was resistant to various antibiotics, including 3rd-generation cephalosporins, penicillins, macrolides, glycopeptides, phenicols (Siedlecka et al., 2021; Furu-hata et al., 2006) and similar resistance pattern was found also for *Afipia* spp. (Siedlecka et al., 2021). Forty percent of the isolates of *Chromobacterium* spp. were resistant to penicillins and aminoglycosides (Jazrawi et al., 1988) and all the tested isolates of *Achromobacter* spp. could be considered resistant to 3rd-generation cephalosporins on the basis of their MIC values (Walsh et al., 2011).

Some studies (5/29) analyzed environmental bacteria as a whole, focusing on HPC flora at 22 °C. For HPC flora, antibiotic-resistance was frequently expressed as percentage comparing the number of colonies counted on nutrient agar supplemented with a certain antibiotic with those counted without the antibiotic (negative control or blank). The presence of antibiotic-resistant HPC was found in many samples of the reviewed studies, with resistance percentage up to 98% for β -lactams (e.g., ampicillin cephalothin, penicillin) (Siedlecka and Piekarska, 2019; Molale-Tom et al., 2024), >59% for 3rd generation cephalosporins (ceftazidime) (Siedlecka et al., 2020), and varying from 10% to 50% for fluoroquinolones (nor-floxacin or ciprofloxacin; Zhang et al., 2018 and Molale-Tom et al., 2024, respectively) and for sulfonamides (sulfamethoxazole or trimethoprim; Zhang et al., 2018 and Ateba et al., 2020, respectively).

Overall, the summarized evidence shows a global interest in understanding the role of environmental bacteria in antibiotic-resistance pathways in tap waters. However, the interpretation of antibiotic-resistance in environmental bacteria strains is still challenging. International committees (EUCAST, CLSI) provide guidelines on antibiotic susceptibility testing only for clinically relevant environmental species (e.g., *Pseudomonas* spp. and *Acinetobacter* spp.) thus they are incomplete for the vast majority of other psychrophilic bacteria. This means that most of the environmental bacteria lack of a methodological standard protocol in terms of, e.g., inoculum preparation, inoculum size, and reading values for MIC or zone diameter breakpoints. Therefore, in the reviewed articles, the Authors frequently adopt breakpoint values established for other species of the same phylum or family or, if they are not available, refer to values already published in literature (e.g., Leginowicz et al., 2018). In some cases, the authors preferred to express only the measured values of MIC, assuming that high MIC values represent patterns of resistance (Furu-hata et al., 2006; Walsh et al., 2011).

3.2.2. Antibiotic-resistance features in fecal ARB and resistant staphylococci

The reviewed articles that found fecal bacteria in tap waters were performed mainly in lower-middle income countries, where a final disinfection by DWTP is not always clearly stated (Table S3) and fecal contamination of tap waters can occur as a result of either heavy pollution of water supplies (that interferes with efficient water treatment) or defects in distribution pipelines that are responsible for post-treatment deterioration of water quality (Ateba et al., 2020; Ahmed et al., 2022; Elmonir et al., 2020; Borjac et al., 2023).

A detailed description of the antibiotic-resistant profile for each fecal ARB is provided in Sect. 3.5 and Table 2, where these bacteria are explored further, given their role in clinical infections. Overall, the total number of resistant isolates in tap waters varies among microorganisms and according to different studies in the literature.

For *E. coli*, the number of resistant isolates ranged from 7.1% (Elmi

et al., 2021) to 36.7% (Kinge et al., 2010), 62.2–66.7% (Ahmed et al., 2022; Jazrawi et al., 1988), and 81.5% (Akbar et al., 2022). The types of resistance was recorded for tetracycline (73–100% of the isolates) (Akbar et al., 2022; Subba et al., 2013; Elmonir et al., 2020), amoxicillin (50–100%) (Akbar et al., 2022; Subba et al., 2013), cephalosporins (67–100%) (Akbar et al., 2022; Elmonir et al., 2020), including 3rd generation ones (35.7%–50%) (Akbar et al., 2022; Subba et al., 2013; Borjac et al., 2023), nalidixic acid (7–87%) (Kinge et al., 2010; Subba et al., 2013; Elmonir et al., 2020; Elmi et al., 2021).

For *Salmonella* spp., Bhatta et al. (2007) reported a very high number of resistant colonies (97.6%). The antibiotic resistance was reported for vancomycin (100%) and erythromycin (100%) (Adzitey et al., 2016), tetracycline (42.9%–100%), ampicillin (100%), chloramphenicol (62.5–100%), trimethoprim-sulfamethoxazol (71.4–100%), nalidixic acid (57.1% for *S. paratyphi* A, 100% *S. tiphymurium* and *S. enteritidis*) (Bhatta et al., 2007).

High resistance levels were observed also for other species of *Enterobacteriaceae* family, such as *Enterobacter cloacae* (65.5%), *Enterobacter agglomerans* (70%), *K. pneumoniae* (75%), and *Serratia odorifera* (53%) (Jazrawi et al., 1988).

Some articles investigated also the presence of *Staphylococcus* spp. The species identified by Borjac et al. (2023) (*S. aureus*, *S. pasteurii*, *S. equorum*) were resistant mainly to sulfonamides (trimethoprim-sulfamethoxazole) (50%), followed by 2nd-generation cephalosporin (cefoxitin) (29%), tetracycline (4%), and aminoglycoside (gentamicin) (4%). Similarly, also Santos et al. (2023) found several species of coagulase-negative staphylococci (e.g., *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. warneri*, *S. condimentii*) resistant to various antibacterial compounds, mainly to sulfonamides (sulfazotrin), macrolides (erythromycin), and penicillin (39%–43% of the tested isolates), but also to cefoxitin, tetracycline, and gentamicin (8%–11%).

3.3. Antibiotic resistant genes

Almost 50% of articles (22/45) studied the presence of ARGs in drinking water, in particular 55% of these manuscripts (12/22) analyzed the presence of ARGs in bacterial strain isolated from tap water (Adesoji et al., 2017; Akbar et al., 2022; Ateba et al., 2020; Borjac et al., 2023; Elmonir et al., 2020; Hamza et al., 2020; Khan and Mustafa, 2021; Khan et al., 2016; Leginowicz et al., 2018; Molale-Tom et al., 2024; Santos et al., 2023; Walsh et al., 2011), while the 45% (10/22) studied the ARG presence directly in water samples (Destiani and Templeton, 2019; Ke et al., 2023, 2024; Li et al., 2023; Liang et al., 2022; Mi et al., 2019; Siedlecka and Piekarska, 2019; Siedlecka et al., 2020; Wang et al., 2023; Zhang et al., 2021a). Fifty-six percent (12/22) considered in parallel also the presence of ARB (Table S3).

3.3.1. ARGs in bacteria isolated from tap water

Considering all the included studies, in 27% (12/45) the investigation of ARGs was carried out from bacterial suspension cultures that have been obtained from tap water concentration and isolation process, that in most of the articles were already tested for phenotypic resistance to a certain antibiotic through culture-based methods (Sect. 3.2), except for two studies (Khan and Mustafa, 2021; Khan et al., 2016).

Such articles were carried out mainly in lower-middle income countries (83% of the reviewed articles), starting from 2010 (Table S3), the mean number of water samples analyzed was 89, and half of the studies considered a high number of water samples ($n \geq 50$) underlining that the data obtained could be a good indicator of the ARG distribution in tap water (Table S3). In 17% of the study (2/12) (Elmonir et al., 2020; Leginowicz et al., 2018) the water volume analyzed was 1 L, while 3 studies analyzed less than 20 mL of samples (25%) (Adesoji et al., 2017;

Molale-Tom et al., 2024; Walsh et al., 2011) and 2 (17%) did not report the volume processed (Akbar et al., 2022; Borjac et al., 2023). Regarding the method used to investigate the ARG presence in isolates, as expected polymerase chain reaction (PCR) assay was the main method performed (11/12) according to that observed by other studies in water environments (Siri et al., 2023). Only in the study by Molale-Tom and collaborators (2024) was the presence of ARGs was monitored using Whole Genome Sequencing (WGS).

In almost all the studies (75%) at least three gene targets were investigated, and the most frequently investigated genes were β -lactamase, in particular *bla*_{CTX-M} gene.

In six articles, the presence of other targets (e.g., sulfonamide resistant genes, tetracycline resistant genes) was deepened (Adesoji et al., 2017; Akbar et al., 2022; Ateba et al., 2020; Khan and Mustafa, 2021; Khan et al., 2016; Molale-Tom et al., 2024). In one article, the target for resistance to quaternary ammonium compounds (*qacS*) was investigated (Khan et al., 2016), while presence of Mobile Genetic Elements (MGEs) (e.g., *intI*, *intII*, *trpA*) was analyzed in four manuscripts (33%) (Adesoji et al., 2017; Khan et al., 2016; Khan and Mustafa, 2021; Hamza et al., 2020). In all studies, bacteria isolated from tap waters hosted one or more ARGs. Overall, the number of isolates that exhibited the ARGs ranged between 8 and 68. Fifty percent of the articles (5/10) showed at least 30% of isolates resistant predominantly the β -lactamase gene *bla*_{CTX-M} (3 out of 12 studies, 25%; Akbar et al., 2022; Elmonir et al., 2020; Hamza et al., 2020) and sulfonamide resistant genes (*sulIII* and *sulII*) (3 out of 12 studies, 25%; Adesoji et al., 2017; Khan et al., 2016; Khan and Mustafa, 2021). The high prevalence of these genes in isolates was confirmed also in drinking water sources (e.g., lake and river) (Reddy et al., 2022; Ana et al., 2021). The similar gene pattern between water supplies and tap water could be related to the lack of effect of drinking water treatments in the dynamics of different ARB.

Considering the MGEs, when they were searched for, they were found by the authors (Akbar et al., 2022; Hamza et al., 2020; Khan et al., 2016; Khan and Mustafa, 2021), according to the results obtained in other studies conducted in Asian water environments (Siri et al., 2023). Considering the drinking water treatments, seven studies report the chlorination as disinfection step utilized for water treatment (Adesoji et al., 2017; Ateba et al., 2020; Borjac et al., 2023; Elmonir et al., 2020; Khan and Mustafa, 2021; Molale-Tom et al., 2024; Santos et al., 2023). Such information underlined that the used treatment seems not to allow an adequate reduction in tap water. It is known that the effect of chlorination on ARGs or ARB can be affected by dosage, nature of chlorination agent, contact time and nature of the ARGs or ARB (Sanganyado and Gwenzi, 2019).

Moreover, it is important to note that in almost all of the studies the resistant isolates were potential opportunistic human pathogens such as *Klebsiella* spp., *E. coli*, *Acinetobacter* spp. These isolates have been frequently associated with infections both in clinical and community settings and could represent a public health threat for susceptible subjects (e.g., hospitalized, immunosuppressed) (OECD, 2023) (see Sect. 3.5 for further information on health-relevance of ARB in the reviewed articles).

3.3.2. ARGs in tap water samples

In these studies (10/45), the investigation of ARGs was performed directly on the microbiota that is retained by filter membranes with micromeritics pore size, thus without any bacterial isolation step.

Such studies were carried out mainly in China (6 out of 10 articles), although one of them also analyzed also few samples from South Africa, USA, Brazil, Taiwan and Singapore (Wang et al., 2023). The other studies were conducted in Poland, Canada and UK. Each article was

published after 2019 (Table S3). All the studies included a number of samples <111, with a mean of 34 samples. The water volume analyzed ranged from 300 to 500 mL up to 2000 L, underlining the variability of volume used for the evaluation. Regarding the methods, all the studies used the filtration method to concentrate the sample for DNA extraction and 80% used a 0.22 μ m membrane (Mi et al., 2019; Siedlecka and Piekarska, 2019; Siedlecka et al., 2020; Zhang et al., 2021a; Liang et al., 2022; Ke et al., 2023, 2024; Li et al., 2023), the 10% used a 0.45 μ m porosity membrane (Destiani and Templeton, 2019) and the 10% (Wang et al., 2023) used both. The filters with the 0.22 μ m pore size were the most used membrane also for the detection of ARGs in other water environments (e.g., wastewater samples) (Milobedzka et al., 2022). In 20% of the reported methods, additional treatments were applied to filters (e.g., washing of the filters to bacterial recovery) (Liang et al., 2022; Wang et al., 2023). The DNA extraction was carried out for all the studies with commercial kit (e.g., DNeasy PowerWater kit Qiagen or FastDNA SPIN Kit for Soil MP biomedical). As expected, the molecular analysis was performed with different approaches, conversely to analysis in tap water isolates. In particular, 50% of the articles used quantitative PCR (qPCR) (Destiani and Templeton, 2019; Mi et al., 2019; Zhang et al., 2021a; Liang et al., 2022; Li et al., 2023), followed by 30% metagenomics (Ke et al., 2023, 2024; Wang et al., 2023) and 20% qualitative PCR (presence/absence) (Siedlecka and Piekarska, 2019; Siedlecka et al., 2020).

Regarding the target of the analysis, a total of 12 types of antimicrobials to which ARGs confer resistance were found (Fig. 4). Along with ARGs, also MGE subtypes were found (MGE were searched together with ARGs in 50% of the reviewed articles; Destiani and Templeton, 2019; Siedlecka and Piekarska, 2019; Siedlecka et al., 2020; Ke et al., 2023; Ke et al., 2024). Besides ARGs and MGEs, also genes related to other resistance mechanisms, such as transmembrane activity and efflux pumps, were investigated in 30% of studies (Siedlecka et al., 2020; Ke et al., 2023, 2024). In general, all the articles investigated at least five ARGs, and the most monitored classes of ARGs were that encoding for β -lactam and sulfonamide (100% of the studies), tetracycline (90%), macrolide and quinolone (80%) resistance, covering a wide range of genes. Other classes were investigated but they were less represented among the reviewed articles (e.g., rifamycins and aminoglycosides). Besides ARGs, in three different studies (30%) the resistance to quaternary ammonium compounds was investigated (Siedlecka and Piekarska, 2019; Siedlecka et al., 2020; Ke et al., 2023). Among the MGEs, instead, the *intI* (integrase) and *trpA* (transposase) were the most investigated genes with a positivity rate of 60% each. Overall, in all the articles at least one ARG was detected in tap water samples. Regarding ARGs, *sulI* (90%), *tetA* (70%), and *bla*_{TEM} (50%) were the most frequently found genes. Interestingly, the ARGs for the β -lactam and sulfonamide resistance are the most frequently detected also in isolates (Sect. 3.3.1). It is possible to observe a strong similarity between ARGs detected in tap water and those in freshwater (Siri et al., 2023). In fact, conventional DWTPs are generally unable to adequately remove ARB or ARGs from water, so if a water supply (e.g., fresh or ground waters) is contaminated by antibiotic-resistance determinants, they can be found also at the exit of the plant.

In fact, in all of the studies that specified the treatment (80%, Destiani and Templeton, 2019; Mi et al., 2019; Siedlecka and Piekarska, 2019; Siedlecka et al., 2020; Liang et al., 2022; Ke et al., 2023; Li et al., 2023; Ke et al., 2024), the final chlorination was not efficient in completely removing the environmental ARGs and MGEs. Moreover, it is important to highlight that the low abatement of ARGs by the chlorination could be related to the disinfection characteristics (e.g., chlorination agent, contact time) (Zhang et al., 2021b).

3.4. Antibiotics in tap waters

Although the detected antibiotics may pose a low risk to human health when considered individually at the residual level in drinking waters, it has become increasingly clear that long term antibiotic exposure could contribute to the evolution of high-level bacterial resistance at concentrations that are several hundred-fold below MICs. Concerning the studies on antibiotic determination in tap waters, screened also taking into account the good practice laboratory and technologies available for the best detection of antibiotics, six studies were considered in the present ScR.

Charuaud et al. (2019) monitored water resources and tap water in an intensive husbandry area in France (Brittany region - northwest France). Authors used both water resource samples collected from 25 sites (23 surface waters and two groundwater) intended for tap water production, and 23 samples from corresponding tap water sites. Thirty-eight veterinary drugs were monitored including 21 antibiotics (amoxicillin, ampicillin, cefquinome, chlortetracycline, doxycycline, enrofloxacin, erythromycin, florfenicol, flumequine, lincomycin, marbofloxacin, neospiramycin, oxolinic acid, oxytetra-cycline, spiramycin, sulfadiazine, sulfadimethoxine, sulfamethazine, tilmicosin, trimethoprim, and tylosin) and 1 antibiotic-metabolite (neospiramycin). Only nine antibiotics were quantified (florfenicol, flumequine, lincomycin, neospiramycin, oxytetracycline, sulfadiazine, sulfamethazine, tilmicosin, trimethoprim) in water samples. Authors found florfenicol which was quantified at 159 ng/L and 211 ng/L and sulfadiazine and tylosin, both in tap waters. As a result of the study, authors reported that 20% of Brittany's tap waters were subject to contamination by residues of veterinary drugs.

In tap waters of Cyprus, Makris and Snyder (2010) screened the presence of both trimethoprim and sulfamethoxazole, and no occurrence of the targeted compounds was found.

In Asiatic countries, especially in China, Hanna et al. (2018) studied the occurrence of norfloxacin, levofloxacin, ciprofloxacin, enrofloxacin, doxycycline, sulfapyridine, sulfamethoxazole, metronidazole, florfenicol, and chloramphenicol residues in waters from Shandong province (eastern China). In the 47 drinking water samples the drugs detected the reported as median concentrations: sulfapyridine (0.5–0.5) ng/L; sulfamethoxazole 1.7 ng/L (0.3–18.6); ciprofloxacin 21.4 ng/L (0.4–224.4); norfloxacin 1.6 ng/L (0.4–3.6); florfenicol 5.4 ng/L (3.3–26.1).

Also, in southern China, Ben et al. (2020) searched for 92 antibiotics in tap waters from the East River (Dongjiang) collected from 10 areas. A total of 58 antibiotics were detected in the filtered tap water and in all

samples chlorotetracycline, tetracycline, 4 epi-tetracycline, doxycycline, oxytetracycline, clarithromycin, midecamycin, roxithromycin, ciprofloxacin, enoxacin, enrofloxacin, norfloxacin, ofloxacin, levofloxacin, N-acetylsulfamethoxazole, sulfadiazine, sulfamethoxazole, sulfamethazine, trimethoprim, monensin, and dicloxacillin were detected. The found compounds ranged from 0.021 ng/L for josamycin to 1133 ng/L for dicloxacillin. Nineteen parent compounds (four tetracyclines, three macrolides, six quinolones, four sulfonamides, one β -lactam, and monensin) and two degradation products (4-epitetracycline and N-acetylsulfamethoxazole) were detected in the 36 filtered tap water samples. The total concentration of the detected antibiotics in such water samples ranged from 6.0 ng/L to 1172 ng/L, and 80 had a total concentration of detected antibiotics of greater than 50 ng/L. Ben's study suggested, hence, a complex antibiotic pollution in Chinese drinking water.

Again, Jiang et al. (2019) studied the occurrence of 43 antibiotics in tap water both from urban and rural areas of a city of the Yangtze River Delta. A minimum of 7 to a maximum of 25 different antibiotics were detected in various types of drinking water with the total concentration ranging from 6.37 ng/L to 809.28 ng/L, frequently including chloramphenicol, quinolones, sulfonamides, macrolides, and lincosamides. The total concentrations of antibiotics in most drinking water were about 100 ng/L or even higher.

Finally, Wang et al. (2011) evaluated the presence of lincomycin, sulfamethoxazole, triclosan, trimethoprim, and tylosin in tap water, where tap water samples were collected from 31 different water treatment facilities of Missouri (USA), and authors showed that antibiotics in tap waters were all below the detection limit. However, in very few samples, lincomycin and sulfamethoxazole were detectable as traces.

3.5. Clinically-relevant ARB in tap waters and public health implication

Some of the reviewed articles (16/45) revealed the presence in tap waters of bacteria, whose antibiotic-resistance profile can pose public health threats, e.g., species of the *Enterobacteriaceae* family, *Enterococcus* genus, *P. aeruginosa*. Some of them belong also to the ESKAPE group, namely nosocomial pathogens that exhibit multidrug resistance and virulence, thus of particular high concern given the circulation among vulnerable subpopulations, where disease outcome may be more severe (Mulani et al., 2019). Clinically-relevant ARB were detected mainly in countries with lower-middle income economies (10/16, 62.5%) and the most representative region according to UNSD division was Asia (7/16, 43.8%). Asian countries (7/16, 43.8%), that include Nepal, India, Pakistan (Fig. 5).

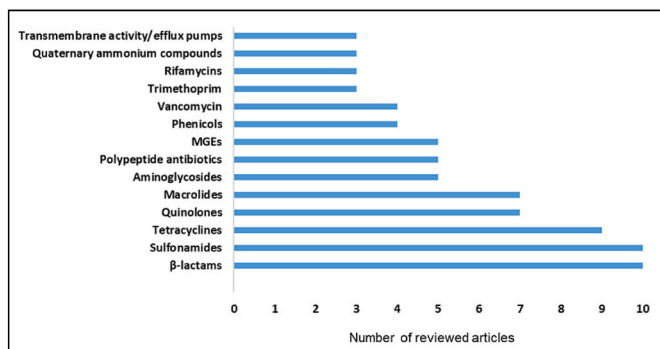


Fig. 4. Occurrence of antibiotic resistance gene (ARG) classes in the reviewed articles. The horizontal axis represents the number of reviewed papers that detected a certain ARG, while the vertical axis lists gene classification, resistance target or mechanisms. MGEs: mobile genetic elements. Polypeptide antibiotics: bacitracin, colistin, polymyxin.

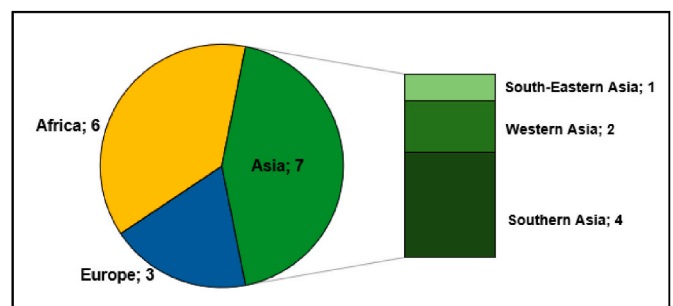


Fig. 5. Geographical location of studies detecting clinically-relevant ARB. The numbers refer to the number of articles published in each area (according to UNSD division; UNSD, 2024).

Table 2

Clinically-relevant ARB detected in tap waters of the revised studies and resistance profile of the analyzed isolates. When only one isolate has been assayed, the profile refers to that specific isolate, thus the percentage was not reported. Antibiotic-resistance profile of concern according to 2024 WHO BPPL are highlighted in bold (WHO, 2024b). References are listed in alphabetical order.

References	WHO-relevant microbes	Type species/group	N. tested isolates	Phenotypic resistance profile of the tested isolates
Adesoji et al. (2017)	Enterobacterales	<i>Citrobacter freundii</i>	1	T, S, AM, SXT, N, AMC, SU
		<i>Proteus vulgaris</i>	2	50% FF, 100% T, 100% S, 100% C, 100% AM, 100% SXT, 100% N, 50% AMC, 100% CEF, 100% SU, 50% G
		<i>Proteus mirabilis</i>	1	T, S, SXT, SU
		<i>Morganella morganii</i>	1	T, S, CEF, AM, SXT, AMC, SU
		<i>Providencia rettgeri</i> ^a	2	50% T, 100% S, 50% C, 50% N, 50% CEF , 50% AM, 100% SXT, 100% AMC, 100% SU
	<i>Acinetobacter baumannii</i>	1	FF, S, C, AM, SXT, AMC, CEF, SU	
Adzitey et al. (2016)	<i>Salmonella</i> spp.		6	100% VA, 100% E
Ahmed et al. (2022)	Enterobacterales	<i>E. coli</i>	82	91.5% CXM, 65.9% SXT, 54.9% AMC, 34.1% CRO , 34.1% C, 17.1% CIP, 15.9% AZ, 6.1% ETP , 3.7% G
	<i>P. aeruginosa</i>		144	52.1% AZ, 11.1% G, 9.7% PTZ, 4.9% CIP
Akbar et al. (2022)	Enterobacterales	<i>E. coli</i>	27	100% T, 100% AM, 100% AMC, 100% CTX , 100% CFX , 75% CAZ , 75% IPM , 75% AMK, 50% G, 50% C, 50% SXT, 50% CIP
Bhatta et al. (2007)	<i>Salmonella</i> spp.	<i>Salm. typhi</i>	16	100% AM, 62.5% C, 75% SXT, 62.5% N , 75% T
		<i>Salm. paratyphi A</i>	7	100% AM, 71.4% C, 71.4% SXT, 57.1% N , 42.9% T
		<i>Salm. typhimurim</i>	16	100% AM, 100% C, 100% SXT, 100% N , 25% CRO
		<i>Salm. enteritidis</i>	2	100% AM, 100% C, 100% SXT, 100% N , 100% CRO
Borjac et al. (2023)	Enterobacterales	<i>E. coli</i>	8	29% AM, 14% FOX, 14% CAZ , 14% AZ,
		Other	5	60% AM, 20% FEP , 100% FOX, 40% CAZ , 20% AZ, 80% CPD , 60% CRO , 20% CTX , 40% SXT
		<i>Enterobacteriaceae</i>	11	9% FEP, 9% CAZ, 18% AZ
	<i>P. aeruginosa</i>		Not specified	21% resistant to cefoxitin as surrogate for methicillin resistance and mecA-positive
Elmi et al. (2021)	Enterobacterales	<i>E. coli</i>	14	7.1% T, 7.1% N, 7.1% AM
Elmonir et al. (2020)	Enterobacterales	<i>E. coli</i> O26:H11 serogroup	12	83.3% S, 83.3% N, 66.6% SXT, 66.6% T, 58.3% CTX , 66.6% C, 25% AM, 33.3% K, 25% CIP, 25% G
		<i>E. coli</i> O103:H2 serogroup	1	AM, CTX, N, CIP, G, S, C, T, SXT
		<i>E. coli</i> O128:H2 serogroup	2	100% AM, 100% CTX , 100% N, 100% 100% CIP, 100% S, 100% C, 100% T, 100% SXT, 100% K
Hamza et al. (2020)	Enterobacterales	<i>E. coli</i>	22	100% FOX , 100% CAZ , 54.5% CTX , 81.8% CRO , 54.5% CRE
		<i>Enterobacter cloacae</i> complex	6	100% FOX , 100% CAZ , 50% CTX , 100% CRO , 50% CRE
Jazrawi et al. (1988)	Enterobacterales	<i>Klebsiella pneumoniae</i>	5	100% FOX , 100% CAZ , 40% CTX , 40% CRO , 40% CRE
		<i>Klebsiella pneumoniae</i>	20	73% AM, 47% CB, 47% CL, 7% K
		<i>Enterobacter cloacae</i>	84	33% AM, 9% CB, 21% CF, 11 CL
		<i>Enterobacter agglomerans</i>	10	71% AM, 43% CB, 57% CF, 11% G, 28% S, 43% SXT
		<i>Citrobacter freundii</i>	21	14% AM, 93% CF, 93% CL
	<i>E. coli</i>	9	50% AM, 50% C, 33% CB, 17% CF, 17% CL, 50% K, 33% S, 17% TE	
	<i>Serratia odorifera</i>	17	22% AM, 22% CB, 11% CF, 11% CL, 33% K, 22% S, 11% SXT	
Kinge et al. (2010)	Enterobacterales	<i>E. coli</i>	60	8.3% K, 31.7% C, 33.3%T, 56.7% AM, 66.7% E, 3.3% S
Leginowicz et al. (2018)	<i>Acinetobacter baumannii</i>		1	AMS
Papandreou et al. (2000)	Enterobacterales	<i>E. coli</i>	10	50% CF, 20% FOX, 30% AM, 100% CB, 30% TC
		<i>Klebsiella pneumoniae</i>	2	100% AM, 100% CB, 100% TC
		<i>Klebsiella oxytoca</i>	1	CF, CXM, FOX, AM, CB
		<i>Enterobacter cloacae</i>	29	96.4% CF, 24.3% CXM, 96.4% FOX, 71.4% AM, 100% CB, 7.1% TC, 22.5% SU, 3.6% C
		<i>Enterobacter agglomerans</i>	9	22.5% CF, 22.5% CXM, 22.5% FOX, 75% AM, 100% CB, 75% TC, 22.5% SU, 22.5% C
		<i>Enterobacter asburiae</i>	3	100% CF, 100% AM, 100% CB, 33.3% TC, 100% SU, 33.3% SXT
		<i>Enterobacter sakazakii</i>	1	CF, FOX, AM, CB, TC
		<i>Citrobacter freundii</i>	4	100% CF, 100% FOX, 100% AM, 100% CB
		<i>Morganella morganii</i>	1	CF, CXM, AM, CB, T, SU, C
		<i>P. aeruginosa</i>	59	100% CF, 93.2% CXM, 98.3% FOX, 86.4% CTX, 67.8% CRO, 100% CTT, 98.3% CZM, 96.6% AM, 13.6% CB, 100% T, 55.9% SU, 22% SXT, 100% C
Siedlecka and Piekarska (2019)	Enterococcus faecium and Enterococcus faecalis		not applicable ^b	VA (5 out of 16 tap water samples showed growth of resistant colonies)
		<i>Klebsiella pneumoniae</i>	not applicable ^c	CRE (2 out of 16 tap water samples showed growth of resistant colonies)
Subba et al. (2013)	Enterobacterales	<i>E. coli</i>	6	100% T, 83% AMX, 50% CFX , 17% AMK, 16.7% N
		Thermotolerant <i>E. coli</i>	8	100% T, 87% AMX, 37.5% CFX , 25% AMK, 25% N
Walsh et al. (2011)	<i>P. aeruginosa</i>		1	CTX, CAZ, IPM , MER , G, AMK, TO, C

Amino-glycosides group: AMK = amikacin; G = gentamicin; K = Kanamycin; S = streptomycin, TO = tobramycin. **Carbapenems group:** CRE = carbapenems (isolates resistant to at least one of the tested carbapenems, namely: Imipenem, meropenem, ertapenem); IPM = imipenem; ETP = ertapenem; MER = meropenem. **Cephalosporin group:** 1st-generation [CED = Cefradine, CF = cephalothin; CFR = cefadroxil]; 2nd-generation [CXM = cefuroxime, FOX = cefoxitin, CTT = cefotetan]; 3rd-generation [CTX = cefotaxime, CRO = ceftriaxone; CZM = ceftizoxime, CEF = Ceftiofur, CAZ = Ceftazidime, CFX = cefixime, CPD = cefpoxime]; 4th-generation [FEP = cefepime]. **(Fluoro)quinolones group:** N = nalidixic acid; CIP = ciprofloxacin. **Penicillin group:** PEN = penicillin; AM = ampicillin; CB = carbenicillin; AMS = ampicillin/sulbactam; AMC = amoxicillin/clavulanic acid; AMX = amoxicillin; TC = ticarcillin; PIP = piperacillin; PTZ = piperacillin-

tazobactam. **Sulfonamides group:** SU = sulfamethoxazole; SXT = trimethoprim-Sulfamethoxazole (or co-trimoxazole). **Others:** C = Chloramphenicol; FF = florfenicol; CL = colistin; OXT = oxytetracycline; T = tetracycline; VA = vancomycin; AZ = aztreonam.

^a 3rd-generation cephalosporin-resistant *Providencia* spp. was included in the 2017 WHO BPPL (WHO, 2017), but it has been removed from 2024 WHO priority list (WHO, 2024b).

^b The assay has been performed on CHROMagar™ VRE, and the results were expressed as CFU/500 ml encountered on chromogenic agar media after incubation.

^c The assay has been performed on CHROMagar™ KPC, and the results were expressed as CFU/500 ml encountered on chromogenic agar media after incubation.

3.5.1. ARB in the priority list of WHO

In the reviewed papers on tap waters, five types of microbes showed resistance patterns of concern according to WHO BPPL, that ranked into critical and high priority groups (Table 2).

- (i) critical group: various species belonging to *Enterobacteriaceae* family (named Enterobacterales in 2024 BPPL), e.g., *E. coli*, *Citrobacter*, *Serratia*, *Morganella*, *Proteus*, *Providencia* (only in 2017 BPPL), *Klebsiella pneumoniae* resistant to carbapenem and/or 3rd gen. cephalosporin (Adesoji et al., 2017; Ahmed et al., 2022; Akbar et al., 2022; Borjac et al., 2023; Elmonir et al., 2020; Hamza et al., 2020; Subba et al., 2013; Siedlecka and Piekarska, 2019);
- (ii) high group: fluoroquinolone-resistant *Salmonella* spp. (Bhatta et al., 2007), carbapenem-resistant *P. aeruginosa* (Walsh et al., 2011), vancomycin-resistant *Enterococcus faecium* (Siedlecka and Piekarska, 2019), and methicillin-resistant *S. aureus* (Borjac et al., 2023).

E. coli of critical priority was widespread in the reviewed articles, mainly resistant to 3rd-generation cephalosporin (e.g., ceftazidime, cefotaxime, cefixime, ceftriaxone, ceftiofur, ceftizoxime, cefpodoxime) but also to carbapenem. In Pakistan, Akbar et al. (2022) found MDR *E. coli* in hospital tap water samples, and all the isolates were resistant to various types of 3rd generation cephalosporin, namely cefotaxime (30 µg/ml) and cefixime (5 µg/ml), and 81.5% to ceftazidime (30 µg/ml); moreover, 81.5% were also resistant to carbapenem (imipenem, 10 µg/ml). Interestingly, similar antibiotic-resistant profiles were also obtained from *E. coli* isolates from clinical samples (urine). In Lebanon, *E. coli* isolates, collected at the exit of domestic water storage tanks, were resistant to ceftazidime, but also to other types of β-lactam compounds (cefoxitin, ampicillin, aztreonam) (Borjac et al., 2023). Also in Egypt, all the serotypes of *E. coli* isolates were resistant to cefotaxime (30 µg/ml); moreover, 7 out of 14 analyzed isolates harbored at least one virulence gene, thus representing an alarming public health threat (Elmonir et al., 2020). Similarly, in Nepal, *E. coli* was resistant to cefixime (5 µg/ml), but also to tetracyclines, fluoroquinolones, and penicillins (Subba et al., 2013). In Ghana, Ahmed et al. (2022) detected MDR *E. coli* of particular concern: 34.1% of the isolates were resistant to ceftriaxone (30 µg/ml) and 6.1% to carbapenem (ertapenem; 10 µg/ml).

Similar antibiotic-resistant profile of critical priority was observed for other species belonging to the *Enterobacteriaceae* family. In fish farm of Egypt, Hamza et al. (2020) tested tap waters used by workers for drinking and hand washing, and they found various *Enterobacteriaceae* (*E. coli*, *Enterococcus*, *K. pneumoniae*) which were all resistant (33 isolates) to 3rd-generation cephalosporin (30 µg/ml) and more than half (51.5%) to carbapenem (10 µg/ml). Resistance of *K. pneumoniae* to carbapenem was observed also in Poland (Siedlecka and Piekarska, 2019). In Nigeria, Adesoji et al. (2017) found several species of *Enterobacteriaceae* (i.e., *Morganella* spp., *Proteus* spp., and *Providencia* spp.) resistant to ceftiofur, 12 µg/ml).

Other authors detected pathogens with resistance pattern of high priority. In particular, in Nepal, Bhatta et al. (2007) found either non-typhoidal and typhoidal *Salmonellae*, that showed resistance to quinolones (nalidixic acid, 10 µg/ml). Then, in India, Walsh et al. (2011) found one isolate of *P. aeruginosa* with MIC values suggesting resistance to carbapenem, in public drinking tap waters. Finally, in Poland, Siedlecka and Piekarska (2019) found 30 colonies of vancomycin-resistant

Enterococcus faecium in 6 out of 16 tap water samples. The same Authors searched also for methicillin-resistant *Staphylococcus aureus* in the same samples, but it was not detected. Conversely, Borjac et al. (2023) detected *S. aureus* resistant to cefoxitin agent, that is frequently used as surrogate marker for the detection of methicillin resistance in this species.

3.5.2. Other clinically-relevant ARB in tap waters

In some studies, the microbes indicated by WHO have been found as MDR, but without the prioritized resistance pattern. In particular, resistance to 3rd-generation cephalosporins and/or carbapenems was tested but not found in *E. coli* isolated from tap water of poultry farms in Malaysia (Elmi et al., 2021) as well as in domestic urban waters in Greece, where also other various MDR *Enterobacteriaceae* were detected (*Enterobacter* spp., *Citrobacter* spp., *Morganella* spp.) (Papandreou et al., 2000) (Table 2).

In other studies, it was not possible to establish if the WHO-relevant species is a priority, because resistance to carbapenems and/or cephalosporin (*Enterobacteriaceae*), carbapenems (*A. baumannii*, *P. aeruginosa*), fluoroquinolone (*Salmonella* spp.) was not tested (Table 2). Nevertheless, *Acinetobacter baumannii* was resistant to various penicillins, sulfonamides and amphenicols in low-middle income country (Nigeria; Adesoji et al., 2017) as well as high-income country (Poland; Leginowicz et al., 2018). Regarding *Enterobacteriaceae*, in Iraq, Jazrawi et al. (1988) reported various MDR isolates mostly resistant to two antibiotic classes widely used at the time of the study, namely aminopenicillins (ampicillin, 10 µg/ml) and 1st-generation cephalosporins (cefalotin, 30 µg/ml). Similarly, in South Africa, Kinge et al. (2010) showed *E. coli* resistance to ampicillin, erythromycin, and chloramphenicol. Regarding *P. aeruginosa*, Ahmed et al. (2022) found most than half of the isolates resistant to monobactam (aztreonam), followed by aminoglycosides (gentamicin) and penicillins (piperacillin-tazobactam), while in Greece *P. aeruginosa* (59 isolates) was resistant up to thirteen antibiotics, with more than 90% of the isolates resistant to 1st-, 2nd-, 3rd-generations cephalosporin, tetracyclines, penicillins, and fluoroquinolones (Papandreou et al., 2000). Also in Lebanon, *P. aeruginosa* isolates were resistant to β-lactams (penicillin and aztreonam) and 3rd-generation cephalosporin, but also to cefpodoxime, a 4th-generation cephalosporin (Borjac et al., 2023).

3.5.3. Possible public health implications

Some of the detected ARB are associated with fecal contamination (*E. coli*, *Salmonella*, *Enterococcus faecium*) and they were detected in tap waters of developing countries. The risk associated to such microorganisms can be reduced by improving water quality safety of the drinking waters, especially with regard to water distribution to households. This aspect is extremely relevant in developing countries, where fecal contamination of drinking waters is quite frequent.

However, antibiotic-resistance has been revealed also in heterotrophic microorganisms, typically living in the environment (e.g., water, soil), such as *Acinetobacter*, *Klebsiella*, *Serratia*, *Pseudomonas*, that commonly occur in drinking waters, e.g., up to 6% of the HPC flora in drinking-water samples is represented by *Acinetobacter* spp. (WHO, 2022b). In fact, they occur in large numbers in raw water sources, then in drinking-water treatment processes can be reduced by coagulation, sedimentation, and disinfection practices, but they can proliferate in biologically active carbon and sand filtration, and growth rapidly in absence of disinfectant residuals (Shi et al., 2013). Moreover, other

microbes belonging to the group of total coliforms, such as the thermotolerant *Klebsiella* and *Citrobacter*, are common in raw waters, and can multiply in the water supply network, especially in the piped distribution system, and may form biofilm (WHO, 2022b).

Heterotrophic microorganisms are traditionally used as indicators of effectiveness of disinfection treatment and cleanliness of distribution system during operational monitoring, but their usefulness in verification and surveillance of water quality is limited because they have little representativeness toward fecal pathogen presence (WHO, 2022b). For this reason, WHO guidelines on drinking water did not release specific regulatory value for this parameter (WHO, 2022b) as well as most of the countries worldwide, as highlighted by a recent overview of national regulations and standards for drinking-water quality (WHO, 2021). As an example, European regulation did not pose specific regulatory values for HPC 22 °C, and reports “no abnormal change” for this parameter (Directive EU, 2020/2184). Nevertheless, in this review, we reported MDR strain of heterotrophic bacteria, with some of them also ranking in critical priority according to 2024 WHO BPPL (WHO, 2024b). Therefore, HPC and environmental strains in tap water, even if they are harmless, could be a threat to human health given their possible role as a reservoir of resistance and ARB dissemination.

3.6. Limitations

Our ScR has some limitations related to the search strategy used during the ScR process. The electronic search was limited to three relevant databases commonly employed in literature reviews on environmental science topics (Pubmed, Scopus, Web of Science), due to the large volume of literature obtained on this topic from these databases. Although there is inadequate evidence to suggesting a specific number of databases or the necessity of including particular databases (Aromataris et al., 2024), limiting the search to a small number of databases could reduce the comprehensiveness of the current evidence on antibiotic-resistance in tap water.

Another limitation is represented by the use of a simple search string. Although this approach was considered appropriate for the focus of the current ScR, which aimed to map available evidence on antibiotic-resistance determinants in tap water, the lack of variations and related terms in the search string could result in an incomplete representation of the relevant articles. Nonetheless, this ScR provided evidence on the breadth of literature in this field of research, thus it can serve as a foundation for future reviews aimed at exploring separately each specific aspect of the phenomenon, namely ARB, ARGs, and antibiotics, by refining and expanding search strategies.

4. Conclusion

Antimicrobial-resistance is recognized as one of the top global public health threats. In the last years, environmental pathways were demonstrated of paramount importance in the development, transmission and spread of the phenomenon. This scoping review provides an overview of antibiotic-resistance in drinking tap water, revealing its potential role as both a reservoir and vehicle of antibiotic-resistant bacteria and their determinants. In particular, the structured literature investigation on this topic allowed to highlight the following aspects.

- I. the presence of multi-drug resistant HPC isolates, regardless of the geographical location, which suggests their environmental role in antibiotic-resistance transmission, despite the HPC parameter being minimally considered in drinking water regulations worldwide;
- II. the presence of clinically relevant ARB in tap water, especially in lower-middle-income economies but also in some European countries;

- III. the presence of ARG, especially those conferring resistance to sulfonamides, tetracyclines and β -lactamases, as well as trace levels of antibiotics.

CRedit authorship contribution statement

Ileana Federigi: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Silvia Bonetta:** Writing – review & editing, Writing – original draft, Formal analysis. **Marina Tesaro:** Writing – review & editing, Writing – original draft, Formal analysis. **Osvalda De Giglio:** Writing – review & editing, Writing – original draft, Formal analysis. **Gea Oliveri Conti:** Writing – review & editing, Writing – original draft, Formal analysis. **Nebiyu Tariku Atomsa:** Formal analysis, Writing – review & editing. **Francesco Bagordo:** Writing – review & editing, Formal analysis. **Sara Bonetta:** Writing – review & editing, Formal analysis. **Michela Consonni:** Writing – review & editing, Formal analysis. **Giusy Diella:** Writing – review & editing, Formal analysis. **Margherita Ferrante:** Writing – review & editing, Formal analysis. **Alfina Grasso:** Writing – review & editing, Formal analysis. **Manuela Macri:** Writing – review & editing, Formal analysis. **Maria Teresa Montagna:** Writing – review & editing, Formal analysis. **Marco Verani:** Writing – review & editing, Formal analysis. **Annalaura Carducci:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2024.120075>.

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