

2019

Groundwater resources as a global reservoir for antimicrobial-resistant bacteria

Luisa Andrade
University College Cork

Madeleine Kelly
Queen's University - Kingston, Ontario

Paul Hynds
Technological University Dublin, Paul.Hynds@tudublin.ie

See next page for additional authors

Follow this and additional works at: <https://arrow.tudublin.ie/ehsiart>



Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Andrade, Luisa; Kelly, Madeleine; Hynds, Paul; Weatherill, John; Majury, Anna; and O'Dwyer, Jean, "Groundwater resources as a global reservoir for antimicrobial-resistant bacteria" (2019). *Articles*. 17. <https://arrow.tudublin.ie/ehsiart/17>

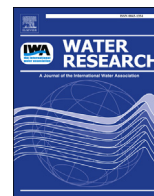
This Article is brought to you for free and open access by the ESHI Publications at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact arrow.admin@tudublin.ie, aisling.coyne@tudublin.ie.



This work is licensed under a [Creative Commons Attribution-NonCommercial-Share Alike 4.0 License](#)

Authors

Luisa Andrade, Madeleine Kelly, Paul Hynds, John Weatherill, Anna Majury, and Jean O'Dwyer



Groundwater resources as a global reservoir for antimicrobial-resistant bacteria

Luisa Andrade^{a, b}, Madeleine Kelly^c, Paul Hynds^{b, d, **, *}, John Weatherill^{a, b, e}, Anna Majury^{c, f}, Jean O'Dwyer^{a, b, e, *}

^a School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland

^b Irish Centre for Research in Applied Geosciences, University College Dublin, Dublin, Ireland

^c Queen's University, Kingston, Ontario, Canada

^d Environmental Sustainability and Health Institute, Technological University Dublin, Dublin 7, Ireland

^e Environmental Research Institute, University College Cork, Cork, Ireland

^f Public Health Ontario, Kingston, Ontario, Canada

ARTICLE INFO

Article history:

Received 23 July 2019

Received in revised form

25 October 2019

Accepted 30 November 2019

Available online 3 December 2019

Keywords:

Groundwater

Antimicrobial resistance

Drinking water

Environment and health

Risk factors

ABSTRACT

Antimicrobial resistance represents one of our most significant global health threats, with increasing incidences noted in both clinical and environmental settings. As such, identifying and understanding the sources and pathways for antimicrobial-resistant bacteria (ARB) is critical. The current study presents the first systematic review and pooled analysis of ARB occurrence in global groundwater supplies, which are used as primary drinking water sources by 2.2 billion people worldwide and are recurrently linked to significant outbreaks of infection. Seventy peer-reviewed studies were identified and included; findings reveal that $80.2\% \pm 29.0$ and $57.2\% \pm 36.8$ of aggregated groundwater isolates were resistant to ≥ 1 and ≥ 3 antimicrobials, respectively. Where bacteria were present, ARB were identified in $76.9\% \pm 33.7$ of individual wells and springs. Our results leave little doubt that groundwater represents a major global reservoir for ARB, however significant research is required to establish environmental determinants and mechanisms mediating their occurrence.

© 2019 Elsevier Ltd. All rights reserved.

1. Introduction

Antimicrobial resistance is now widely recognised as a global public health threat, requiring multi-sectorial preventative and mitigative interventions (Bradford and Harvey, 2017; WHO, 2018; Larsson et al., 2018). Anthropogenic influences and behaviours, including the misuse/overuse of human and veterinary antimicrobials has resulted in the addition of significant selective pressures to the naturally occurring resistance within and between bacterial species (Van Boeckel et al., 2014; Bell et al., 2014; O'Neill, 2016). As such, a global increase in acquired resistance traits has been noted among bacterial isolates, including clinically significant species (e.g. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and

Enterobacteriaceae spp.; WHO, 2017) with the incidence of multi-drug resistance (i.e., resistance to ≥ 3 antimicrobials) increasing both spatially and temporally (Munita and Arias, 2016). Cases of treatment failure of both human and veterinary infectious diseases are increasingly documented (Wright, 2010; Opatowski et al., 2019), which results in higher healthcare costs, more severe and prolonged infections, and rising rates of morbidity and mortality (Laxminarayan et al., 2013). Recent estimates by the European Antimicrobial Resistance Surveillance Network (EARS-Net) indicate that approximately 33,000 deaths are attributable to antimicrobial-resistant bacterial infections per annum within the EU/EEA, comparable with the combined human health burden of influenza, tuberculosis and HIV/AIDS in the same region (Cassini et al., 2019). Accordingly, antimicrobial resistant infections have received significant attention within both the media and research community over the past two decades, with an extensive body of research existing in clinical settings, coinciding with the development of several antimicrobial resistance Action Plans at varying scales (European Commission, 2011; WHO, 2015). More recently, however, the role of the natural aquatic environment as a source and

* Corresponding author. School of Biological, Earth and Environmental Sciences, University College Cork, Cork, Ireland.

** Corresponding author. Environmental Sustainability and Health Institute, Technological University Dublin, Dublin 7, Ireland

E-mail address: Jean.ODwyer@ucc.ie (J. O'Dwyer).

transmission pathway for antimicrobial-resistant bacteria (ARB) has been acknowledged as an area of growing concern (Sanderson et al., 2018).

Human and veterinary antimicrobials are increasingly released to the environment at sub-therapeutic concentrations via myriad sources of domestic, agricultural, industrial and clinical/hospital origin. These microbiologically diverse, pharmaceutically dilute media may readily catalyse the development of ARB within the natural environment, inevitably resulting in their ingress to both surface and groundwater sources (Van Schaik, 2015). However, at present, more research is still needed to better understand the occurrence and transport of ARB to and in natural waterbodies. This is particularly true with regard to groundwater wells and aquifers, which currently supply approximately 31.5% (2.2 billion people) of the global population with domestic drinking water (Murphy et al., 2017).

Microbial contamination of groundwater and its adverse public health effects have been well substantiated within the scientific literature. A recent review by Murphy et al. (2017) presents clear epidemiological evidence of disease transmission due to groundwater contamination at a global scale, with an estimated 35.2 to 59.4 million cases of acute gastrointestinal infection potentially attributable to groundwater consumption per year. Thus, the potential implications of groundwater-borne ARB pose a significant threat to public health, allied with an already high global burden of infection. A recent study in the Republic of Ireland found that wastewater systems, livestock density and the presence of children in a household were significantly associated with the presence of antimicrobial resistant *Escherichia coli* (*E. coli*) in private groundwater supplies (O'Dwyer et al., 2017). As such, the ubiquity of contaminant sources, in concurrence with the presence of bacteria and sub-therapeutic antimicrobial concentrations, suggests that vulnerable groundwater systems may be a significant and frequently overlooked reservoir for ARB (Wellington et al., 2013). Indeed, research carried out with 878 Canadian individuals has shown that consumers of *E. coli* contaminated groundwater are 1.26 times more likely to be colonised by antimicrobial resistant *E. coli* than non-consumers (Coleman et al., 2012). Nonetheless, there is presently no consensus regarding the role of groundwater in the global dissemination of ARB. The extent of this threat is further complicated by the nuances of groundwater contamination mechanisms, which are typically determined and/or driven by numerous environmental and source-specific risk factors (e.g. source design, location and maintenance, local hydrogeological setting, and shifting climatic and landuse patterns), their permutations and spatiotemporal distributions (Hynds et al., 2012; Wallender et al., 2014; Atherholt et al., 2017; Andrade et al., 2018). Accordingly, the current study sought to further understand the occurrence, distribution and potential drivers of ARB in groundwater sources (i.e. wells and springs) via a global pooled analysis of peer-reviewed studies. Findings can be used to support evidence-based risk management strategies to inform non-clinical

considerations in existing antimicrobial resistance Action Plans and guide future research strategies.

2. Methods

2.1. Literature identification

Identification of scientific articles examining the occurrence of ARB in groundwater sources was conducted with an overarching systematic review protocol based upon pre-established guidelines (Pullin et al., 2018) and adapted from previous studies (Eftim et al., 2017; Nappier et al., 2019). Defined search terms (Table 1) were used to search Scopus, Web of Science, Pub Med and ProQuest databases on October 8th, 2018. Employed "Outcome" search terms, and particularly the antimicrobials and bacterial species selected, were based on the global priority list of ARB (WHO, 2017). Manual supplementary searches were additionally performed between October 15th and November 12th, 2018. These comprised the examination of article bibliographies and studies citing articles which were identified via database search and marked as "provisionally included" upon title and abstract assessment (n = 215).

2.2. Study selection

Studies uncovered during the identification phase (i.e. database and supplementary searches) were independently screened by two authors using explicit eligibility criteria (Table 2) via title and abstract assessment. Articles without an available full text were excluded, with eligibility disagreements resolved via provisional inclusion. All "provisionally included" and manually identified studies had full-texts manually (i.e., no computer-assisted techniques employed) and independently assessed (eligibility assessment) using the presented criteria (Table 2). Disagreements at this stage were resolved via author panel consensus.

Excluded studies were those that: (i) reviewed previously published studies, (ii) incorporated controlled elements in their study design (i.e., spiking or laboratory-based (micro-, meso-) groundwater environments), (iii) examined thermal/hot springs, (iv) did not study water samples derived from groundwater sources (e.g., surface and marine water), (v) combined resistance data from multiple environmental media, (vi) did not examine ≥ 10 groundwater samples (including articles where sampling number was not reported), (vii) did not analyse antimicrobial resistance/susceptibility in bacterial isolates from groundwater (including studies where bacteria were not found), (viii) did not report number of isolates tested, (ix) did not report bacterial resistance (or susceptibility) to each antimicrobial tested against (Fig. 1).

2.3. Study inclusion

During full-text assessment (eligibility phase), studies included were those that assessed 10 or more groundwater samples and

Table 1
Search terms employed in the current study.

Element	Description	Search terms
Population	All non-saline groundwater	Groundwater, Ground Water, Aquifer, Subsoil, Subsurface, Borehole, Bore Well, Bored Well, Dug Well, Well Water, Water Well
Outcome(s)	Occurrence of antimicrobial-resistant (or susceptible) bacteria	Antimicrobial, Antibiotic, Antibacterial, Bacteriostatic, Bactericidal, Penicillin, Cephalosporin, Carbapenem, (Fluoro)quinolone, β lactam, Aminoglycoside, Tetracycline, Vancomycin, Clarithromycin, Ampicillin, Sulphonamide AND Resistance, Resistant, AND Bacteria, Bacterial, Microbe, Microbial, Organism, Susceptible, Susceptibility, Sensitive, Sensitivity Pathogen, <i>E. coli</i> , <i>Escherichia coli</i> , <i>Pseudomonas</i> , <i>Enterococcus</i> , <i>Enterococci</i> , <i>Campylobacter</i> , <i>Salmonella</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Shigella</i> , <i>Klebsiella</i> , <i>Enterobacter</i> , <i>Enterobacteriaceae</i>

Table 2
Eligibility (inclusion/exclusion) criteria employed.

Inclusion Criteria	Exclusion Criteria
<p>Study type: All peer-reviewed articles excluding reviews</p> <p>Language: English</p> <p>Population: Naturally occurring groundwater environments; groundwater sources (i.e. wells and springs)</p> <p>Exposure: Pre-existing environmental exposures (i.e. prior to study)</p> <p>Event/Outcome: Antimicrobial-resistant (or susceptible) bacteria found in groundwater resources</p> <p>Study design: Analysis of ≥ 10 groundwater samples; results including percentages of bacterial isolates resistant/susceptible to each antimicrobial tested against.</p> <p>Period: Any</p>	<p>Study type: Academic reviews; grey literature</p> <p>Language: non-English</p> <p>Population: Artificial groundwater media (i.e. lab-based); pre-packaged water; Thermal/Hot springs; surface water bodies; maritime aquatic environments; wastewater treatment plants; soil; saline, brackish, or soil water.</p> <p>Exposure: Any controlled exposure (i.e. spiking)</p> <p>Event/Outcome: Absence of bacterial contamination in groundwater; absence of antimicrobial resistance profiling of groundwater isolates</p> <p>Study design: Analysis of < 10 groundwater samples (includes number of groundwater samples not reported); results combined for different sampled environments; results combined for all antimicrobials tested against; number of isolates tested not reported.</p> <p>Period:</p>

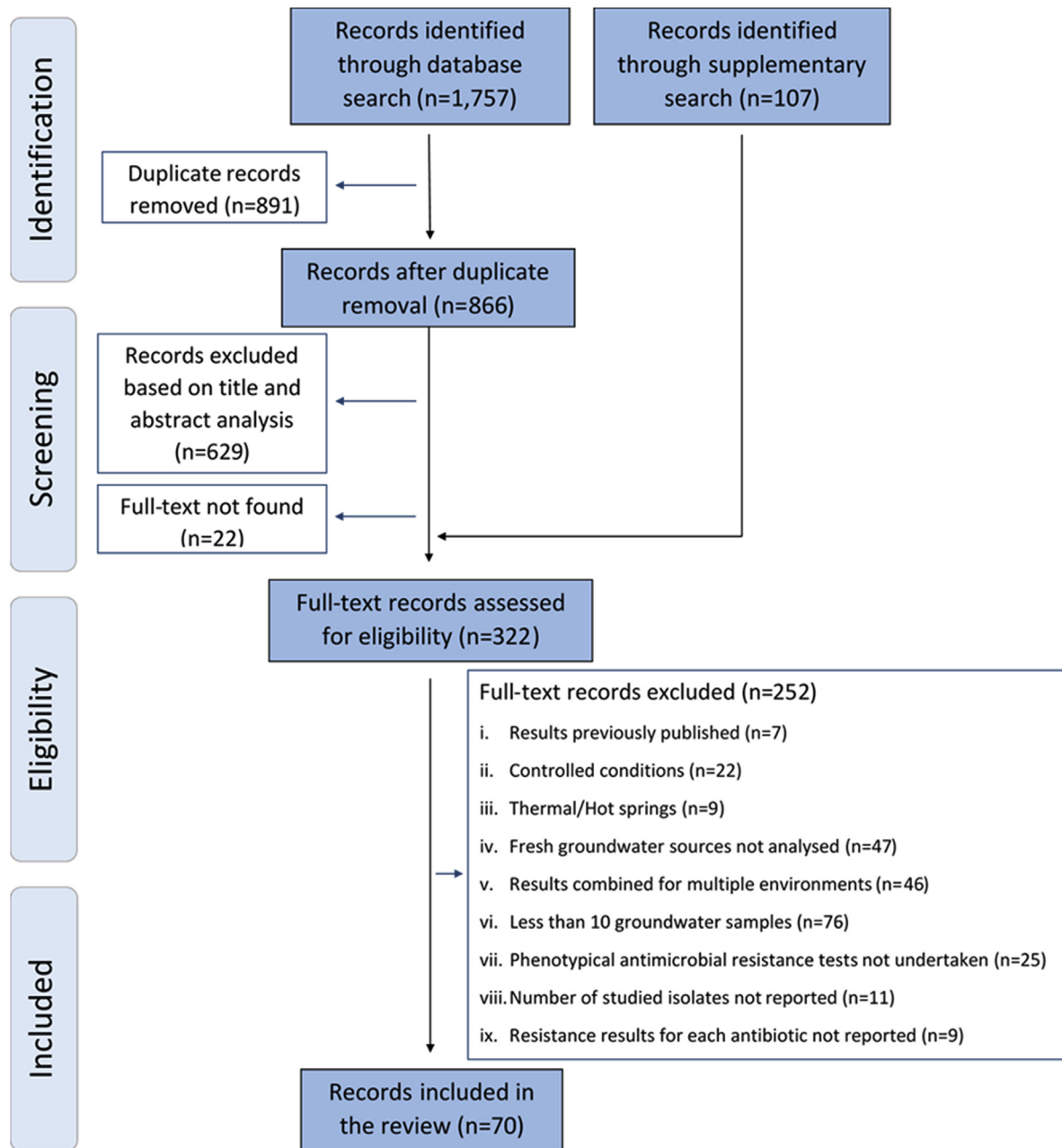


Fig. 1. Systematic review protocol employed during the current study, including literature identification, screening, eligibility assessment, and final study inclusion.

explicitly stated the percentage of bacterial isolates resistant or susceptible to each antimicrobial agent tested against. In all, 76 studies were excluded due to reporting on < 10 groundwater samples, including studies where sample number was not reported ($n = 12$) (exclusion criteria vi). Forty-six studies reported composite occurrence rates of ARB in combined study environments (e.g., merged findings from groundwater, surface water and/or wastewater), and as such were excluded under exclusion criteria (v). Studies that did not provide an adequate description of water sample origin; that is, referred solely to “tap water” ($n = 14$) were excluded under exclusion criteria (iv) (i.e. groundwater source not analysed) and where bacterial isolates were not identified in groundwater samples ($n = 4$), articles were excluded under exclusion criteria (vii), as antimicrobial resistance could not be determined, this criteria also comprised studies that provided an assessment of antimicrobial resistance through genotypical methods only (i.e. presence/absence of resistance genes via qPCR or digital droplet qPCR analyses). As such, 70 of 1864 identified studies (identification phase) were deemed eligible for inclusion following the full review process (Fig. 1).

2.4. Critical appraisal of study validity

Included articles were independently evaluated by two authors using a critical appraisal tool adapted from Bain et al. (2014). In it, a score ranging from 0 to 14 was attributed to each study according to the number of affirmative responses to the fourteen pre-established criteria. Based on it, articles were classified as presenting low (score ≤ 5), medium (score of 6–8) or high (score ≥ 9) validity. Individual article assessments are presented in Supplementary Table 1. All disagreements were resolved by a consensus between authors.

2.5. Data extraction

Relevant data pertaining to each included study were extracted to MS Excel 2016. Extracted variables were classified and exported under six primary categories; namely, bibliographic details, study region (e.g., country, location within country and settlement type), groundwater characteristics (e.g., source type, well type, ownership, uses and treatment presence), sampling regime (e.g., number of samples, re-sampling, length of sampling regime), analytic elements (e.g. bacterial species tested, antimicrobial agents tested against, method and criteria were used to assess susceptibility/resistance), and resistance profile (e.g., percentage of ARB and MRB amongst tested isolates and percentage of resistance to each antimicrobial agent tested against). It is important to note that with regards to studies in which re-sampling was employed, data pertaining to different sampling rounds were merged and extracted as single outcomes, with “re-sampling” and “one-off” used just to classify two contrasting approaches employed across identified studies. As each study only provides one outcome to the analysis, independence between observations from each analytical unit (i.e. study) can be assumed. Moreover, due to the large periodicity associated with repeat groundwater sampling rounds (e.g. where groundwater sources are sampled many months apart), observations may be treated independently due to the fluid (acute) nature of groundwater contamination (Bjerg and Christensen, 1992; Morvan et al., 2006; Pacheco Castro et al., 2018).

Antimicrobial resistance results were extracted according to the standards (i.e. EUCAST, CLSI, etc.) and interpretations employed in each published manuscript, inhibition zone and/or minimal inhibitory concentration results were not routinely specified and as such, could not be uniformly re-assessed. Moreover, as intermediate resistance indicates that an antimicrobial is ineffective at

recommended and commonly used therapeutic concentrations (Rodloff et al., 2008), potentially resulting in treatment failure, it was considered as resistance, as per other studies (Reinthal et al., 2003; O'Dwyer et al., 2017).

Where key variables were unclear or not explicitly documented, article authors were contacted for clarification and/or articles were analysed for identifiable characteristics, and thus classified. Where classification was not possible, variables were categorised as “not reported”. Sample sizes were categorised as small (<30 samples), medium (30–99 samples) or large (≥ 100), as previously defined by Bain et al. (2014), with a maximum threshold established whereby data from one study comprising > 5000 samples were not extracted to prevent geographical and/or analytical bias (Coleman et al., 2013); however, data reported from a smaller sample ($n = 657$) in this study were included in analyses. As such, the maximum sample number from a single included study was 939 (Akoachere et al., 2013).

Studies were further classified according to globally established characteristics relating to the study regions. Countries were classified as low, lower-middle, upper-middle, and high income based upon World Bank classification (World Bank, 2018). Specific study areas' primary (arid, cold, polar, temperate and tropical) and secondary (e.g., dry summer, dry winter, without dry season, monsoon and rainforest) climates were determined based on the Köppen-Geiger climate classification (Peel et al., 2007), and used to ascertain sampling season (i.e., summer, winter, spring, autumn) and period (i.e., wet or dry).

Moreover, where possible, occurrence rates of antimicrobial resistance (≥ 1 antimicrobial) and multidrug resistance (≥ 3 antimicrobials) were calculated for all isolates reported in a study (i.e. number of resistant and multidrug resistant isolated, respectively, divided by the total number of isolates recovered from all groundwater sources and samples examined within that study). This approach was used as even when studies employed re-sampling in their methodology (i.e. more than one sample taken from the same groundwater sources at different times), antimicrobial resistance results were often integrated during reporting. With regards to occurrence rates of ARB amongst groundwater sources (i.e. wells and/or springs) or the specific sources in which bacterial isolates were found, these comprised the percentage of sources that harboured ARB at least once, as reported in each manuscript (i.e. number of sources where ARB were found at least once divided by total number of tested sources or by the number of sources in which bacteria were found at least once, respectively).

2.6. Multiple antimicrobial resistance index

Multiple Antimicrobial Resistance (MAR) indices were calculated to standardise the rates of antimicrobial resistance reported across each study (Equation (1); Krumperman, 1983). MAR indices provide a single measure of antimicrobial resistance and control for the number of antimicrobial agents tested against, thus avoiding potential bias (i.e. elevated ARB occurrence rates are typically found when more antimicrobials are incorporated in a study design).

$$MAR \text{ index} = \frac{y}{n \times x} \quad [\text{Equation. 1}]$$

where y is the aggregate antimicrobial resistance score of all isolates tested (i.e., the sum of isolates resistant to each antimicrobial), n is the number of isolates tested, and x is the number of antimicrobial agents tested against (Krumperman, 1983).

A single MAR index was calculated for each study, irrespective of temporal methodologies employed, and included in the analyses. These were used to ascertain the overall findings regarding

antimicrobial resistance in groundwater bacteria on a study by study basis, thus permitting cross-study comparisons (see Fig. 5). Secondly, for descriptive purposes only, discrete MAR indices were calculated for each study within isolates of the same species or genus and within different antimicrobial classes. These were not included in analyses, but merely used to (a) identify the relative rates of antimicrobial resistance associated with each bacterial genus, and (b) determine the differences between resistance rates within and between different antimicrobial classes (see Table 5).

2.7. Data analyses

Three dependent variables were calculated and used to quantify presence of ARB and/or Multidrug Resistant Bacteria (MRB) in groundwater and represent the main findings from each included study (i.e. studies were the analytic unit throughout analyses), namely: (i) occurrence rates of antimicrobial resistance (≥ 1 antimicrobial) amongst all groundwater isolates tested; (ii) occurrence rates of multidrug resistance (≥ 3 antimicrobials) amongst all groundwater isolates tested; and (iii) calculated study-specific MAR indices.

Dependent variables were not normally distributed and could not be normalized using standard (transformation) techniques, thus non-parametric Mann-Whitney U and Kruskal-Wallis tests were employed to identify categorical associations between dependant and independent variables (i.e. the extracted characteristics outlined in Table 3). Where significance was found ($p < 0.05$) and the independent variable described three or more levels of measurement, Dunn's (non-parametric) pairwise post-hoc tests were used. Mean MAR indices were further calculated for each country and discretized into ranges (e.g. 0.000–0.100, 0.100–0.200, ...) using all included studies, with all studies equally weighted.

3. Results

3.1. External, groundwater-specific and study-specific characteristics

A total of 70 relevant studies were included for data extraction and pooled analyses. A summary of all included studies is presented in Table 4, with detailed study characteristics and full reference list provided in Supplementary Materials 2. Relevant studies were

conducted in geographically, climatically and economically diverse regions and spanned a 42-year period (1976–2018), with 81.4% ($n = 57$) of identified articles published since 2010. All inhabited continents as well as four of the five climatic zones were represented. Overall, 51.4% ($n = 36$) of identified studies were undertaken in lower-middle income countries, followed by countries classified by high (21.4%; $n = 15$) and upper-middle (21.4%; $n = 15$) incomes. There were two primary sampling regimes identified, with repeat (temporal/seasonal) sampling employed in 32.9% ($n = 23$) of studies, while 52.9% ($n = 37$) performed one-off ('snapshot') sampling. Where sampling seasons could be ascertained ($n = 58$), it was exclusively undertaken during wet and dry seasons in 41.4% ($n = 24$) and 32.7% ($n = 19$) of studies, respectively; however, just 10.3% of studies ($n = 6$) explicitly reported this information.

Overall, 52.9% ($n = 37$) of studies were conducted in categorically rural areas, while 27.1% ($n = 19$) were undertaken in urban regions and the remaining 5.7% ($n = 4$) in "mixed settlements" (i.e. both urban and rural areas surveyed). Approximately two thirds (64.3%; $n = 45$) of studies reported the presence of waste sources adjacent to study sites; 79.2% ($n = 38$) of human and 48.9% ($n = 22$) of animal origin (e.g. septic tanks, wastewater treatment plants, animal grazing fields and manure application). Wells and springs were examined in 91.4% ($n = 64$) and 12.9% ($n = 9$) of included studies, respectively, with 4.3% ($n = 3$) reporting concurrent analyses of both source types. Well construction was explicitly defined in 23 studies, of which 60.9% ($n = 14$) were hand-dug and 39.1% ($n = 9$) were bored. A significant paucity of data was encountered with respect to reporting of numerous source-specific elements (e.g. supply depth, age and operational condition/performance), with just 18.6% ($n = 13$) of included studies describing one or more of these. Similarly, just 17.1% ($n = 12$) of studies reported local hydrogeological characteristics (e.g. aquifer type, subsoil type, depth and permeability, bedrock geology and groundwater vulnerability). Sampled groundwater sources were used for human consumption in 57 of the 60 studies where groundwater usage was reported (95%), with an absence of water treatment before consumption noted in 76.9% of the 13 studies that reported this information ($n = 10$).

A total of 8741 groundwater samples were collected across all included studies, ranging from 10 to 939 per study (mean \pm SD = 125 \pm 189), with 7157 identified groundwater

Table 3

External, source-specific and study-specific characteristics and their sub-categories used as independent variables in the non-parametric statistical tests.

Categories	Sub-categories	Non-parametric test Sub
Environmental		
1. Economic classification ^a	Low; Lower middle; Upper middle; High income	Kruskal-Wallis
2. Climate ^b	Tropical; Arid; Cold; Temperate	Kruskal-Wallis
3. Sampling period ^c	Wet; Dry	Mann-Whitney U
4. Settlement type ^d	Urban; Rural	Mann-Whitney U
5. Waste source adjacent to study site ^e	Human waste; Animal waste	Mann-Whitney U
Source-specific		
6. Source type	Well; Spring	Mann-Whitney U
7. Well type	Hand-dug; Bored	Mann-Whitney U
Study-specific		
8. Sampling regime	One-off sampling; Re-sampled sources	Mann-Whitney U
9. Sample size	Under 30; 30 to 99; 100 and over	Kruskal-Wallis
10. Length of sampling period	Under 6 months; 6–12 months; more than 12 months	Kruskal-Wallis

^a Economic classification according to the World Bank (2018).

^b Climate classified according to Peel et al. (2007).

^c Wet and dry as defined in Peel et al. (2007).

^d Urban settlements include urban, sub-urban or peri-urban regions.

^e Human waste encompasses septic tanks and wastewater treatment plants, and animal waste encompasses animal grazing fields and agricultural fields where manure is spread.

Table 4
Summary of principal characteristics extracted, with study (n = 70) and ground-water sample (n = 8741) number associated with each corresponding sub-category.

Characteristics	Studies	Samples
	n (%)	n (%)
Publication year		
Pre-1990	6 (8.6)	446 (5.1)
1990–1999	0 (0.0)	0 (0.0)
2000–2009	7 (10.0)	696 (8.0)
2010–2018	57 (81.4)	7599 (86.9)
Continent		
Africa	32 (45.7)	3941 (45.1)
Asia	22 (31.4)	2645 (30.3)
Central and South America	6 (8.6)	436 (5.0)
North America	4 (5.7)	1032 (11.8)
Europe	5 (7.1)	658 (7.5)
Oceania	1 (1.4)	29 (0.3)
Economic classification^a		
Low income	3 (4.3)	149 (1.7)
Lower-middle income	36 (51.4)	4466 (51.1)
Upper-middle income	15 (21.4)	1194 (13.7)
High income	16 (22.9)	2932 (33.5)
Climate^b		
Tropical	25 (35.7)	3506 (40.1)
Arid	15 (21.4)	850 (9.7)
Temperate	24 (34.3)	3193 (36.5)
Cold	6 (8.6)	1192 (13.6)
Polar	0 (0.0)	0 (0.0)
Sampling period^c		
Wet	24 (34.3)	2677 (30.6)
Dry	19 (27.1)	1185 (13.6)
Both	15 (21.4)	3857 (44.1)
Not identifiable	12 (17.1)	1022 (11.7)
Sample size^d		
Small (<30)	13 (18.6)	–
Medium (30–99)	34 (48.6)	–
Large (≥100)	23 (32.8)	–
Length of sampling period		
0–6 months	23 (32.9)	1511 (17.3)
7–12 months	15 (21.4)	3009 (34.4)
> 12 months	6 (8.6)	1907 (21.8)
Not reported	26 (37.1)	2314 (26.5)
Sampling regime		
One-off sampling	37 (52.9)	2920 (33.4)
Re-sampled sources	23 (32.9)	4405 (50.4)
Not reported	10 (14.3)	1416 (16.2)
Validity score		
Low (≤5)	5 (7.1)	245 (2.8)
Medium (6–8)	19 (27.1)	2377 (27.2)
High (≥9)	46 (65.7)	6119 (70.0)
Source type		
Wells	61 (87.1)	7981 (91.3)
Dug	13 [21.3]	2123 [26.6]
Bored	9 [14.8]	1236 [15.5]
Mixed	9 [14.8]	1526 [19.1]
Not reported	30 [49.1]	3096 [38.8]
Springs	6 (8.6)	423 (4.8)
Mixed	3 (4.3)	337 (3.9)
Water supply		
Private	17 (24.3)	2893 (33.1)
Public	1 (1.4)	108 (1.2)
Mixed	2 (2.9)	275 (3.1)
Not reported	50 (71.4)	5465 (62.5)
Primary settlement type^e		
Rural	37 (52.9)	4328 (49.5)
Urban	19 (27.1)	2858 (32.7)
Mixed	4 (5.7)	975 (11.2)
Not reported	10 (14.3)	580 (6.6)

Table 4 (continued)

Characteristics	Studies	Samples
	n (%)	n (%)
Waste adjacent to GW sites^f		
Human waste	23 (32.9)	3301 (37.8)
Animal waste	7 (10.0)	1326 (15.2)
Both animal and human waste	15 (21.4)	2489 (28.5)
Not reported	25 (35.7)	1625 (18.6)
Treatment before use		
Yes	3 (4.3)	223 (2.6)
No	11 (15.7)	1027 (11.7)
Mixed ^g	4 (5.7)	1680 (19.2)
Not reported	52 (74.3)	5811 (66.5)
Human consumption		
Yes	57 (81.4)	7036 (80.5)
No	3 (4.3)	299 (3.4)
Not reported	10 (14.3)	1406 (16.1)
Antimicrobials tested against		
1–5	2 (2.9)	145 (1.7)
6–10	35 (50.0)	4660 (53.3)
11–15	20 (28.6)	2589 (29.6)
16–20	13 (18.6)	1347 (15.4)
Type of bacteria		
Gram-positive	8 (11.4)	461 (5.3)
Gram-negative	49 (70.0)	6412 (73.4)
Both	12 (17.1)	1793 (20.5)
Not specified	1 (1.4)	75 (0.9)

^a Countries economically classified according to the [Work Bank \(2018\)](#).

^b Climate classification according to [Peel et al. \(2007\)](#).

^c As defined in [Peel et al. \(2007\)](#).

^d Sample size classified following approach in [Bain et al. \(2014\)](#).

^e Urban settlements include urban, sub-urban and/or peri-urban regions.

^f Human waste encompasses septic tanks and wastewater treatment plants, and animal waste encompasses animal grazing fields, agricultural fields where manure is spread, etc.

^g "Mixed" treatment before use encompasses studies in which treated and untreated supplies were combined during analysis.

isolates examined for resistance against 89 distinct antimicrobial agents. *E. coli* was the most frequently analysed bacteria (54.3% of studies; n = 38), followed by *Pseudomonas* spp. (21.4%; n = 15). Combined, these corresponded to almost half of the isolates tested across all studies, with 2737 and 824 tested isolates, respectively. The Penicillin antimicrobial class was most frequently incorporated in study designs (94.3%; n = 66), followed by Aminoglycosides (87.1%; n = 61), Fluoroquinolones (81.4%; n = 57), Cephalosporins (72.9%; n = 51), and Tetracyclines (71.4%; n = 50).

3.2. Critical appraisal of study validity

Study validity scores obtained varied greatly among included studies, spanning from 5 to 13, however most studies were considered of medium to high quality (i.e. ≥6; 92.8%; n = 65). The frequency of studies in each quality category are summarized in [Table 3](#) and complete analysis can be found in Supplementary Materials.

3.3. Antimicrobial-resistant bacteria in global groundwater

Where reported (n = 20 studies; 28.6%), ARB were identified in 31.4% ± 32.6 of studied groundwater sources and in 76.9% ± 33.7 of sources where bacteria were present. Additionally, 80.2% ± 29.0 and 57.2% ± 36.8 of pooled groundwater isolates (3456 and 1403 isolates, respectively) were resistant to ≥ 1 (ARB; n = 55; 78.6%) and ≥3 antimicrobials (MRB; n = 32; 45.7%), respectively.

Table 5
Summary statistics of MAR indices calculated within antimicrobial classes and bacterial species.

Bacteria	Aminoglycosides ¹	Carbapenems ²	Cephalosporins				Fluoroquinolones ³	Glycopeptides ⁴	Lincosamides ⁵	Macrolides ¹⁰	Penicillins ¹¹	Sulfonamides ¹²	Tetracyclines ¹³	TOTAL				
			1st gen ⁸		2nd gen ⁴										3rd gen ⁵		4th gen ⁶	
			mean ± SD (n)	mean ± SD (n)	mean ± SD (n)	mean ± SD (n)									mean ± SD (n)	mean ± SD (n)	mean ± SD (n)	mean ± SD (n)
Gram-positive																		
<i>Enterococcus</i> spp.	0.344 ± 0.268 (7)	-	0.333 ± 0.333 (2)	0.923 ± 0.000 (1)	0.615 ± 0.440 (3)	-	0.188 ± 0.190 (6)	0.433 ± 0.350 (7)	0.692 ± 0.000 (1)	0.466 ± 0.245 (8)	0.518 ± 0.302 (10)	0.600 ± 0.400 (2)	0.417 ± 0.262 (9)	0.396 ± 0.198 (11)				
<i>Staphylococcus</i> spp.	0.250 ± 0.314 (10)	-	0.451 ± 0.399 (3)	0.380 ± 0.302 (3)	0.283 ± 0.349 (7)	-	0.270 ± 0.390 (7)	0.285 ± 0.184 (3)	0.424 ± 0.348 (5)	0.208 ± 0.273 (4)	0.350 ± 0.255 (10)	0.629 ± 0.000 (1)	0.293 ± 0.194 (7)	0.334 ± 0.235 (11)				
<i>Bacillus</i> spp.	0.243 ± 0.388 (5)	-	0.286 ± 0.286 (2)	0.583 ± 0.083 (2)	0.322 ± 0.349 (4)	-	0.333 ± 0.471 (3)	-	0.889 ± 0.079 (3)	0.545 ± 0.455 (2)	0.554 ± 0.246 (5)	0.000 ± 0.000 (1)	0.071 ± 0.071 (2)	0.443 ± 0.163 (5)				
<i>Streptococcus</i> spp.	0.544 ± 0.390 (3)	-	-	0.683 ± 0.111 (2)	0.646 ± 0.256 (3)	-	0.545 ± 0.386 (3)	0.606 ± 0.000 (1)	0.714 ± 0.000 (1)	0.412 ± 0.412 (2)	0.561 ± 0.174 (4) ^a	0.861 ± 0.000 (1)	0.585 ± 0.068 (3)	0.581 ± 0.146 (4)				
<i>Micrococcus</i> spp.	0.406 ± 0.406 (2)	-	-	0.469 ± 0.000 (1)	0.328 ± 0.328 (2)	-	0.422 ± 0.422 (2)	-	0.813 ± 0.000 (1)	0.000 ± 0.000 (1)	0.286 ± 0.286 (2)	-	0.000 ± 0.000 (1)	0.332 ± 0.332 (2)				
Gram-negative																		
<i>Escherichia coli</i>	0.270 ± 0.302 (35)	0.051 ± 0.107 (7) ^a	0.599 ± 0.284 (5)	0.421 ± 0.333 (15)	0.375 ± 0.391 (19) ^a	0.036 ± 0.055 (4)	0.212 ± 0.226 (34)	0.486 ± 0.367 (3)	-	0.402 ± 0.288 (4)	0.486 ± 0.335 (36)	0.394 ± 0.281 (8)	0.428 ± 0.277 (28)	0.339 ± 0.226 (38)				
<i>Pseudomonas</i> spp.	0.322 ± 0.371 (15)	0.351 ± 0.157 (4) ^a	0.950 ± 0.000 (1)	0.666 ± 0.356 (6)	0.478 ± 0.380 (10) ^a	0.185 ± 0.185 (2)	0.332 ± 0.371 (13)	0.194 ± 0.000 (1)	-	0.750 ± 0.204 (3)	0.614 ± 0.298 (15)	0.780 ± 0.137 (4)	0.643 ± 0.342 (10)	0.544 ± 0.237 (15)				
<i>Klebsiella</i> spp.	0.307 ± 0.321 (12)	0.000 ± 0.000 (1) ^a	0.586 ± 0.268 (3)	0.428 ± 0.384 (5)	0.261 ± 0.360 (7) ^a	0.000 ± 0.000 (1)	0.260 ± 0.358 (11)	0.080 ± 0.080 (2)	-	0.750 ± 0.250 (2)	0.666 ± 0.229 (12)	0.956 ± 0.000 (1)	0.442 ± 0.285 (8)	0.423 ± 0.231 (12)				
<i>Shigella</i> spp.	0.329 ± 0.406 (10)	0.000 ± 0.000 (1) ^a	0.788 ± 0.226 (3)	0.484 ± 0.358 (5)	0.276 ± 0.246 (6) ^a	0.000 ± 0.000 (1)	0.272 ± 0.367 (9)	-	-	0.542 ± 0.208 (2)	0.591 ± 0.212 (10)	0.857 ± 0.000 (1)	0.404 ± 0.342 (6)	0.424 ± 0.229 (10)				
<i>Salmonella</i> spp.	0.300 ± 0.353 (9)	0.000 ± 0.000 (1) ^a	0.902 ± 0.000 (1)	1.000 ± 0.000 (1)	0.338 ± 0.413 (7) ^a	-	0.134 ± 0.246 (9) ^a	1.000 ± 0.000 (2)	-	0.802 ± 0.217 (3)	0.577 ± 0.410 (10)	0.427 ± 0.357 (3)	0.308 ± 0.372 (8)	0.391 ± 0.271 (10)				
<i>Aeromonas</i> spp.	0.011 ± 0.018 (7)	0.000 ± 0.000 (2)	0.586 ± 0.309 (3)	0.813 ± 0.000 (1)	0.159 ± 0.268 (5)	0.000 ± 0.000 (1)	0.000 ± 0.000 (6)	1.000 ± 0.000 (1)	1.000 ± 0.000 (1)	0.778 ± 0.314 (3)	0.730 ± 0.331 (7)	1.000 ± 0.000 (1)	0.100 ± 0.200 (5)	0.304 ± 0.135 (7)				
<i>Proteus</i> spp.	0.384 ± 0.432 (7)	-	0.555 ± 0.016 (2)	0.524 ± 0.278 (4)	0.494 ± 0.271 (4) ^a	-	0.366 ± 0.317 (6)	-	-	0.500 ± 0.000 (1)	0.524 ± 0.274 (7)	1.000 ± 0.000 (1)	0.683 ± 0.331 (4)	0.471 ± 0.182 (7)				
<i>Acinetobacter</i> spp.	0.117 ± 0.194 (5)	0.000 ± 0.000 (1) ^a	0.467 ± 0.033 (2)	0.708 ± 0.257 (3)	0.594 ± 0.427 (4) ^a	0.000 ± 0.000 (1)	0.263 ± 0.327 (4)	-	0.750 ± 0.000 (1)	1.000 ± 0.000 (1)	0.563 ± 0.343 (5)	0.833 ± 0.000 (1)	0.000 ± 0.000 (2)	0.388 ± 0.151 (5)				
<i>Citrobacter</i> spp.	0.000 ± 0.000 (5)	0.000 ± 0.000 (1)	0.180 ± 0.055 (2)	0.563 ± 0.438 (2)	0.250 ± 0.433 (4)	0.000 ± 0.000 (1)	0.000 ± 0.000 (4)	-	-	0.625 ± 0.000 (1)	0.505 ± 0.346 (5)	0.588 ± 0.000 (1)	0.123 ± 0.102 (3)	0.269 ± 0.155 (5)				
<i>Serratia</i> spp.	0.200 ± 0.400 (5)	0.333 ± 0.000 (1) ^a	0.536 ± 0.036 (2)	0.667 ± 0.333 (2)	0.111 ± 0.157 (3) ^a	0.000 ± 0.000 (1)	0.167 ± 0.289 (4)	-	-	0.250 ± 0.250 (2)	0.652 ± 0.129 (5)	0.429 ± 0.000 (1)	0.000 ± 0.000 (3)	0.351 ± 0.138 (5)				
<i>Vibrio</i> spp.	0.359 ± 0.178 (4)	-	-	0.415 ± 0.000 (1)	0.360 ± 0.454 (3)	-	0.000 ± 0.000 (2)	1.000 ± 0.000 (1)	-	0.590 ± 0.410 (2)	0.820 ± 0.312 (4)	0.875 ± 0.125 (2)	0.491 ± 0.425 (4)	0.520 ± 0.237 (4)				
<i>Alcaligenes</i> spp.	0.389 ± 0.437 (3)	-	0.750 ± 0.000 (1)	0.500 ± 0.000 (1)	0.250 ± 0.250 (2)	-	0.375 ± 0.375 (2)	-	-	0.000 ± 0.000 (1)	0.757 ± 0.236 (3)	0.417 ± 0.000 (1)	0.250 ± 0.250 (2)	0.461 ± 0.205 (3)				
<i>Flavobacterium</i> spp.	0.250 ± 0.204 (3)	-	0.500 ± 0.000 (1)	0.500 ± 0.000 (1)	0.500 ± 0.500 (2)	-	0.250 ± 0.250 (2)	-	0.750 ± 0.000 (1)	1.000 ± 0.000 (1)	0.611 ± 0.283 (3)	0.333 ± 0.000 (1)	0.000 ± 0.000 (2)	0.397 ± 0.282 (3)				
<i>Shigella</i> spp.	0.567 ± 0.419 (3)	-	0.833 ± 0.000 (1)	0.458 ± 0.458 (2)	-	-	0.375 ± 0.375 (2)	-	-	1.000 ± 0.000 (1)	0.617 ± 0.238 (3)	1.000 ± 0.000 (1)	0.500 ± 0.500 (2)	0.607 ± 0.273 (3)				
<i>Chromobacterium</i> spp.	0.000 ± 0.000 (2)	1.000 ± 0.000 (1)	0.667 ± 0.000 (1)	-	0.500 ± 0.000 (1)	0.000 ± 0.000 (1)	0.000 ± 0.000 (1)	-	-	0.750 ± 0.250 (2)	0.000 ± 0.000 (1)	0.000 ± 0.000 (1)	0.000 ± 0.000 (1)	0.339 ± 0.106 (2)				
TOTAL¹⁴	0.253 ± 0.274 (61)	0.040 ± 0.078 (16)	0.594 ± 0.325 (14)	0.529 ± 0.316 (26)	0.326 ± 0.291 (38)	0.150 ± 0.236 (8)	0.178 ± 0.199 (57)	0.549 ± 0.342 (15)	0.493 ± 0.325 (7)	0.493 ± 0.325 (21)	0.492 ± 0.313 (66)	0.517 ± 0.315 (18)	0.393 ± 0.285 (50)	0.352 ± 0.207 (70)				

MAR Index = Multiple antimicrobial resistance index = aggregate antimicrobial resistance score of all isolates tested / (number of isolates tested × number of antimicrobials tested against) (Krumperman, 1983). = mean MAR index ≤ 0.200; = mean MAR index from 0.201 to 0.400; = mean MAR index from 0.401 to 0.600; = mean MAR index from 0.601 to 0.800, and = mean MAR index from 0.801 to 1.000. SD = Standard deviation, n = number of studies.

¹ Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Paromomycin, Streptomycin and/or Tobramycin; 61 studies, ² Bismepem, Ertapenem, Imipenem and/or Meropenem; 16 studies, ³ Cefadroxil, Cefalexin, Cefazolin, Ceftriaxone and/or Cefuroxime; 26 studies, ⁴ Ceftazidime, Ceftaxime, Cefoperazone, Cefoperazone-sulbactam, Cefotaxime, Cefotaxime-clavulanic acid, Ceftiposolime, Cefuroxime, Cefuroxime, Cefepime; 8 studies, ⁵ Clindamycin and/or Vancomycin; 15 studies, ⁶ Clindamycin and/or Lincomycin; 7 studies, ⁷ Erythromycin and/or Roxithromycin; 21 studies, ⁸ Amoxicillin, Amoxicillin-clavulanic acid, Ampicillin, Ampicillin-sulbactam, Ampiclox, Carbapenem, Cloxacillin, Cloxacillin, Meclizine, Methicillin, Oxacillin, Piperacillin, Piperacillin-tazobactam, Ticarcillin and/or Ticarcillin-clavulanic acid; 66 studies, ⁹ Sulfamethoxazole, Sulfanilamide, Sulfasalazine and/or Sulfabenzamide-sulfathiazole (i.e. "Triple Sulfas"); 18 studies, ¹⁰ Chlorotetracycline, Doxycycline, Oxytetracycline and/or Tetracycline; 50 studies.

^a Priority 1 (i.e. critical) in the global priority list of antimicrobial-resistant bacteria⁷

^b Priority 2 (i.e. high) in the global priority list of antimicrobial-resistant bacteria⁷

^c Priority 3 (i.e. medium) in the global priority list of antimicrobial-resistant bacteria⁷

^d The only *Pseudomonas*, *Klebsiella* and *Acinetobacter* species included in the global critical priority list of antimicrobial-resistant bacteria are *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*, respectively⁷

^e The only *Streptococcus* species included in the global medium priority list of antimicrobial-resistant bacteria is *Streptococcus pneumoniae*⁷

^f The total calculated MAR Indices were obtained per study, amalgamating results for all bacteria tested (included ones of unspecified genus) and using Equation 1. All studies were weighted equally

As shown (Fig. 2), consistently high occurrence rates of antimicrobial resistance were found among *Pseudomonas* (99.9% ± 0.2), *Klebsiella* (99.8% ± 0.6) and *Enterobacter* spp. (99.2% ± 2.2), as well as others which were reported in fewer studies (i.e. <7), with *Pseudomonas* spp. also exhibiting high rates of multidrug resistance (96.6% ± 5.8). Moreover, *Pseudomonas* spp. (0.544 ± 0.237) were associated with some of the highest calculated MAR indices, across the 15 studies that examined the genus (Fig. 2). MAR indices calculated within antimicrobial classes (Table 5) indicate that 1st generation Cephalosporins (0.594 ± 0.325), Glycopeptides (0.549 ± 0.378), 2nd generation Cephalosporins (0.529 ± 0.316) and Sulphonamides (0.517 ± 0.315) were associated with the highest mean MAR Indices. Conversely, lowest mean values were associated with Carbapenems (0.040 ± 0.078) and 4th generation Cephalosporins (0.150 ± 0.236).

A calculated mean MAR index of 0.352 ± 0.207 represents the level of ARB presence in global groundwater during the total review period (i.e. between 1976 and 2018; Fig. 3), and of 0.359 ± 0.207 from 2010 to 2018 (not shown). Just one study exhibited a calculated MAR index of 0.000 (zero) (i.e. Traoré et al., 2015). MAR index values were highest in Kenya (x ± SD = 0.549 ± 0.208), Nepal (0.545), China (0.537 ± 0.041), Morocco (0.528), Saudi Arabia (0.516) and South Africa (0.513 ± 0.122). At a continental level, MAR indices were highest in Africa (0.423 ± 0.202) and Asia (0.370 ± 0.201), followed by Central and South America (0.281 ± 0.098), Oceania (0.173 ± 0.000), Europe (0.138 ± 0.083) and North America (0.110 ± 0.045).

3.4. Potential drivers of antimicrobial-resistant bacteria in groundwater

As shown (Fig. 4), occurrence rates of antimicrobial resistance in groundwater bacteria were significantly higher in urban

settlements (p = 0.020), with rates of MRB in groundwater from high income countries significantly lower than those from upper-middle income countries (p = 0.035). Studies that employed one-off sampling regimes yielded significantly higher percentages of both antimicrobial and multidrug resistance in groundwater bacteria when compared to regimes where re-sampling was employed (p = 0.001 and p = 0.002, respectively).

MAR indices were significantly lower in groundwater from high-income countries when compared to upper-middle (p = 0.018) and lower-middle (p = 0.002) income countries (Fig. 5), and significantly higher in arid versus temperate regions (p = 0.021) and in samples collected during dry versus wet seasons (p = 0.014). Significantly higher MAR indices were also observed in urban settlements (p = 0.001) and when waste sources adjacent to groundwater supplies were predominantly of human (e.g. septic tanks and wastewater treatment plants) as opposed to animal (e.g. animal grazing and manure spreading) origin (p = 0.005). No significant differences were found when comparing MAR index distributions with sources type (i.e. wells versus springs) or source construction (i.e. hand-dug versus bored wells). One-off sampling regimes (n = 37) yielded significantly higher MAR indices in groundwater when compared to regimes where re-sampling was employed (n = 23) (p = 0.003).

4. Discussion

Antimicrobial resistance is a widely recognised global public health threat with growing evidence of its spread beyond clinical settings now available within the scientific literature (Bradford and Harvey, 2017; Larsson et al., 2018; Opatowski et al., 2019). Most recently, the role of the natural aquatic environment in the dissemination of ARB has gained interest (Suzuki et al., 2017; Sanderson et al., 2018), with groundwater resources being

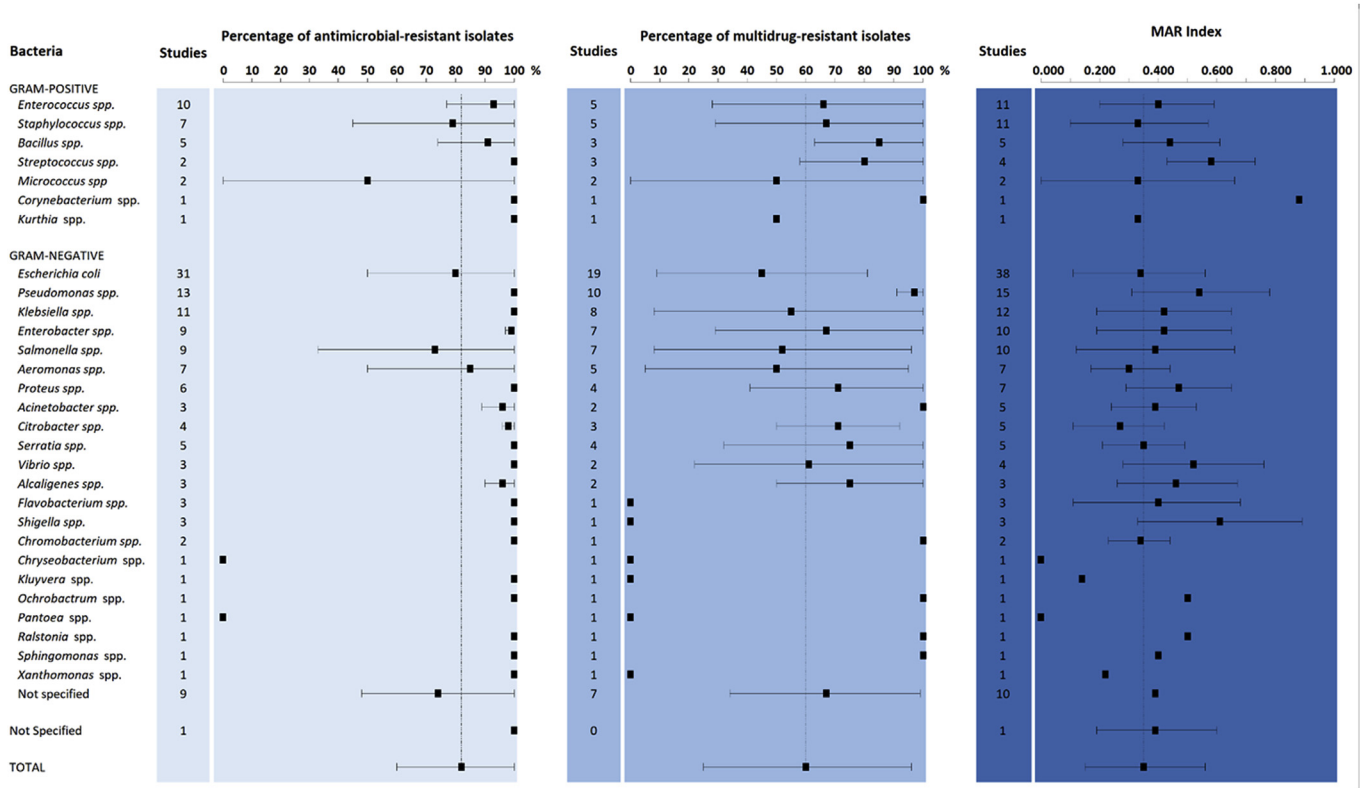


Fig. 2. Forest plot of (i) antimicrobial resistance, (ii) multidrug resistance and (iii) calculated multiple antimicrobial resistance (MAR) Indices in groundwater isolates from each genus examined.

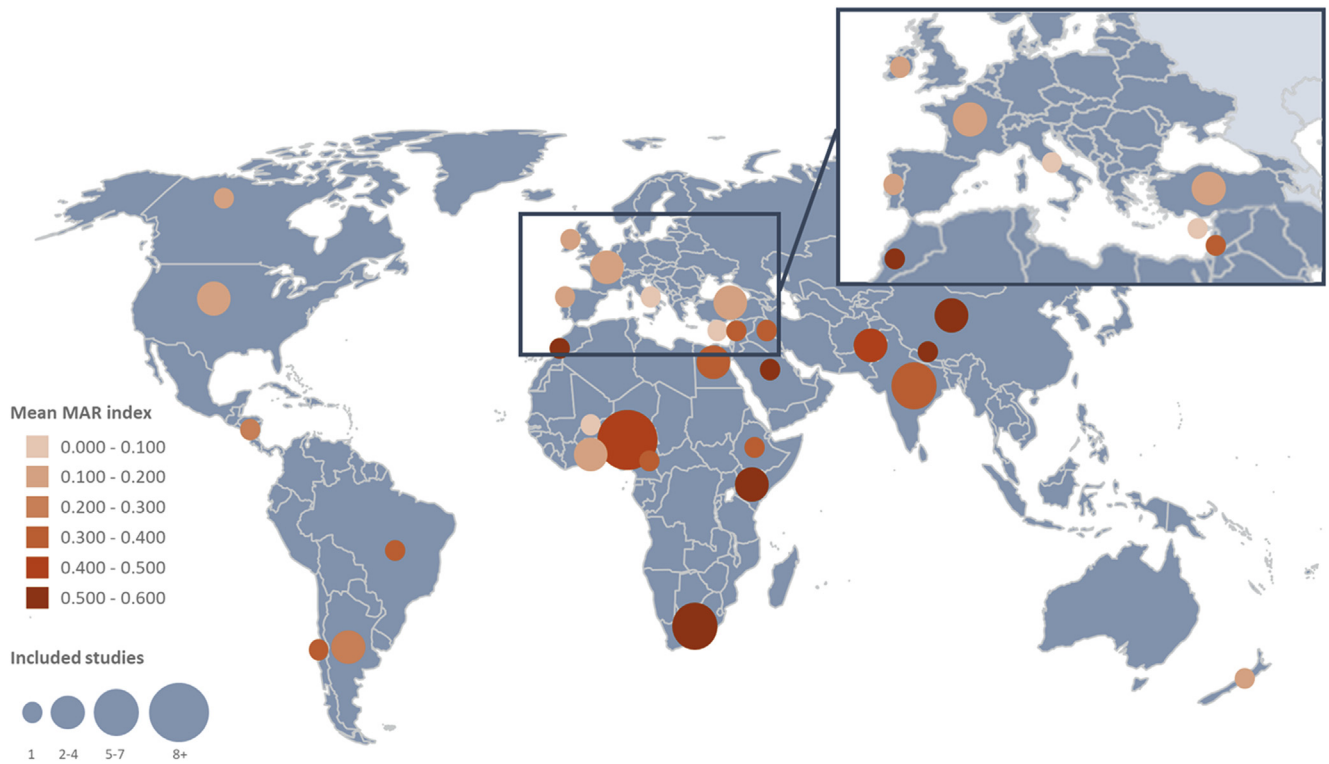


Fig. 3. Mean Multiple Antimicrobial Resistance (MAR) Indices in global groundwater from 1976 to 2018 (n = 70) in each included country.

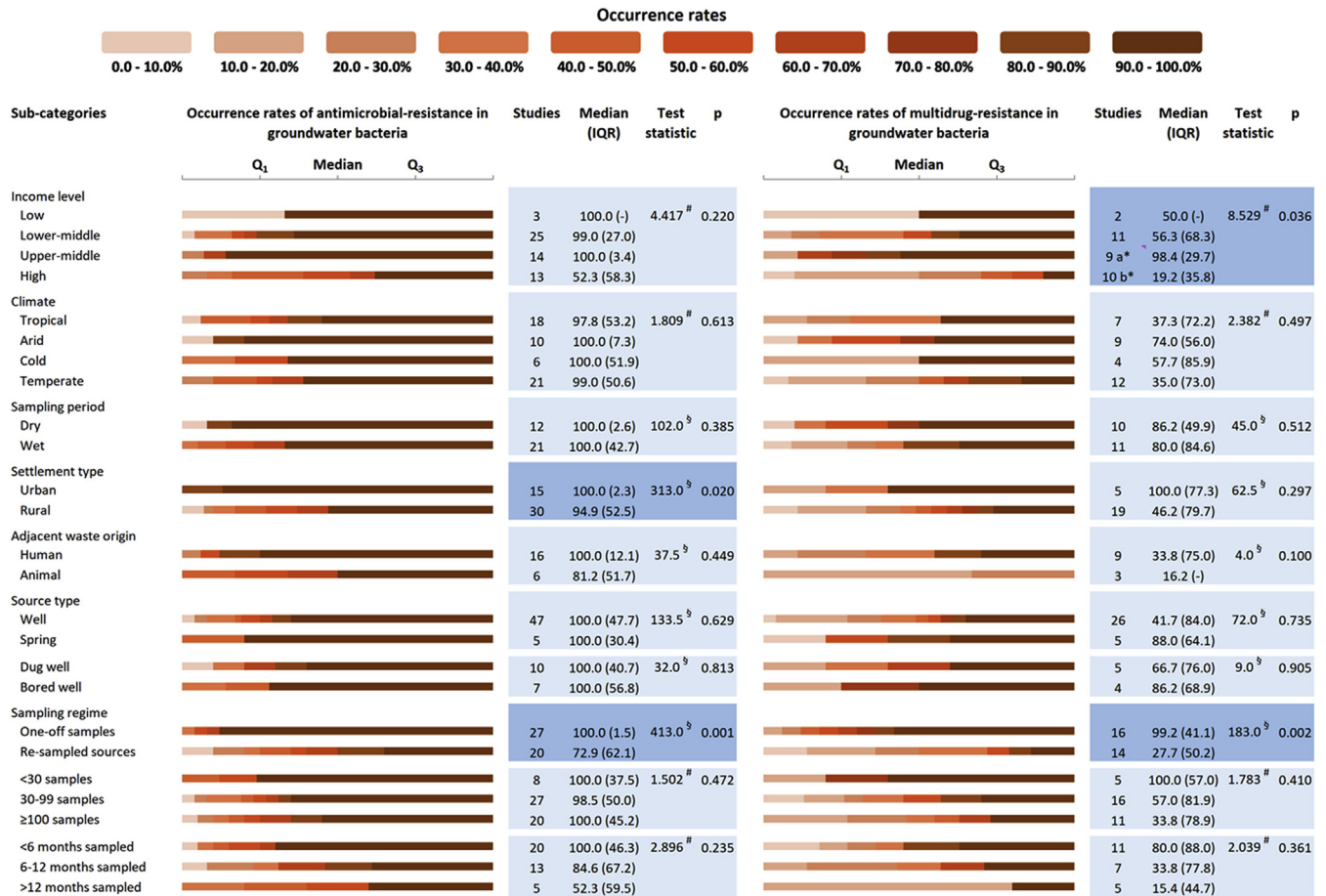


Fig. 4. Summary of descriptive and non-parametric test results for occurrence rates of (i) antimicrobial (n = 55) and (ii) multidrug resistance (n = 32) amongst groundwater isolates tested. Q₁ = 25th percentile; Q₃ = 75th percentile; IQR = Interquartile range = Q₃ - Q₁; # p ≥ 0.1; § p < 0.05; # Kruskal-Wallis test; § Mann-Whitney U test; * Dunn's pairwise tests (each similar letter denotes a subset of each category whose distribution do not differ significantly from each other at the p < 0.05 level and different letters indicate statistically significant differences at the p < 0.05 level).

particularly relevant given the reliance of 2.2 billion people around the world on this source of drinking water (Murphy et al., 2017). Moreover, due to the widely held belief that groundwater is an intrinsically 'clean' water source, many sources lack treatment, in spite of well document incidences of faecal contamination as a result of agricultural and wastewater sources (Schets et al., 2005; Hynds et al., 2014; Murphy et al., 2017). As such, the risks posed via consumption of contaminated groundwater are significant, with a recent United Nations (UN) report referring to discharge of contaminated wastes to the environment and inadequate access to clean water as key drivers of antimicrobial resistance (IACC, 2019). However, despite the importance of groundwater for human consumption and its potential role in the global resistome, to date no comprehensive synthesis of previous studies of ARB in the sub-surface has been undertaken. The current study sought to address this gap in the scientific literature.

Occurrence rates of ARB amongst groundwater-derived isolates were consistently high across the seventy included studies, which were undertaken in geographic, climatologic and socioeconomically diverse regions (Fig. 2; Table 4). In the pooled analysis, studies where isolates were not obtained were excluded and hence our findings are relevant to groundwater sources where bacteria are present, which, where reported, accounted for 31.4% ± 32.6 of studied groundwater sources. Results show that four fifths (80.2% ± 29.0) of aggregated groundwater isolates were resistant to one or more antimicrobial agents, with ARB identified in

76.9% ± 33.7 of individual groundwater sources where bacteria were present. These findings seem to suggest that in the minority of groundwater sources where bacteria were present they were often resistant to at least one antimicrobial. Just one study, undertaken in Burkina Faso, reported no ARB in examined groundwater sources (Traoré et al., 2015). The high mean MAR index values calculated across differing regions (Fig. 3), when considered in concurrence with high occurrence rates of MRB (57.2% ± 36.8), further highlights the presence of multidrug resistance as a particular issue within groundwater sources. Compounding this, myriad ARB listed on the World Health Organisation priority list as 'critical' (WHO, 2017) were specifically noted in across included studies (Table 5); a high proportion of which were used for human consumption (e.g. Ribeiro et al., 2014; Maran et al., 2016; O'Dwyer et al., 2017). Accordingly, pooled results point to groundwater as an environment characterised by the presence of ARB and MRB, and highlight its potential role in the spread of antimicrobial resistance both within the environment and directly to humans via consumption of, frequently untreated, drinking water (Coleman et al., 2012).

Similarly, the abovementioned concurrently high incidence of ARB and MRB, and elevated pooled mean MAR index (0.352 ± 0.207), suggests the presence of significant selective pressures within groundwater systems, potentially resulting from extended residence times along subsurface pathways with exposure to sub-therapeutic concentrations of antimicrobial residues. Continuous release of human and veterinary antimicrobials to the

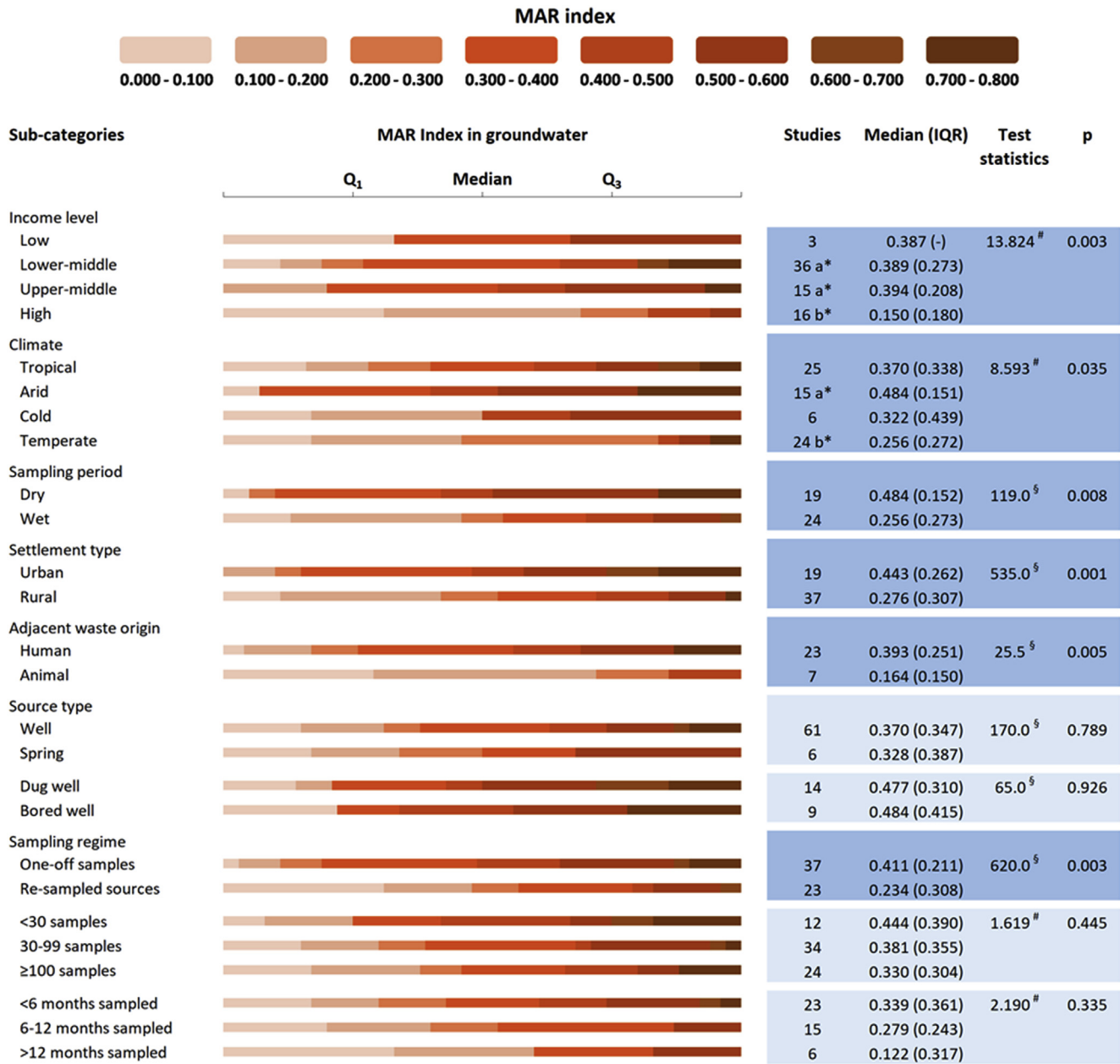


Fig. 5. Summary of descriptive and non-parametric test results for the calculated Multiple Antimicrobial Resistance (MAR) indices in groundwater (iii; n = 70). Q₁ = 25th percentile; Q₃ = 75th percentile; IQR = Interquartile range = Q₃ - Q₁; [§] p ≥ 0.05; [#] p < 0.05; [#] Kruskal-Wallis test; [§] Mann-Whitney U test; * Dunn's pairwise tests (each similar letter denotes a subset of each category whose distribution do not differ significantly from each other at the p < 0.05 level and different letters indicate statistically significant differences at the p < 0.05 level).

natural environment has been shown to facilitate their ingress to both surface and groundwater sources (Van Schaik, 2015); however, occurrence rates of antimicrobial resistance in surface water bacteria found in middle- and high-income countries (O'Flaherty and Cummins, 2017) are lower than those encountered in the current study. Unlike surface water, extended residence times associated with subsurface systems may lead to prolonged bacterial exposure to antimicrobial residues (at low concentrations), resistance genes and ARB within a relatively confined and oftentimes buffered (e.g. UV, pH and temperature) environment (Van Schaik, 2015; Williams-Nguyen et al., 2016). Compounding this, bacterial isolates may present high rates of resistance prior to subsurface ingress, with a recent study reporting that tetracycline-resistant

E. coli strains were more mobile than susceptible strains in saturated porous media (Walczak et al., 2011), and thus can be characterised by higher rates of transport in the subsurface. However, there is limited research exploring this hypothesis in diverse soil and aquifer types. Indeed, further research is required which combines microbial source tracking, advanced hydrogeological modelling, antimicrobial susceptibility testing and pharmaceutical residue concentrations to facilitate a greater understanding of the intricacies of microbial transport and resistance acquisition by these microbes in the subsurface.

More generally, pooled analyses suggest a paucity of research specifically addressing the mechanisms mediating the occurrence of ARB and MRB in groundwater, particularly with respect to

varying climates, anthropogenic practices, hydrogeological settings, and groundwater source types. This represents a common limitation within blended groundwater and microbiological studies; due to their multidisciplinary nature, important source-pathway-receptor information are oftentimes omitted, overlooked or unreported (Hynds et al., 2014). For example, just 8.6%, 17.1% and 18.6% of included studies explicitly reported meteorological, hydrogeological, and detailed source-specific data, respectively. Accordingly, in combination with the inherently high degree of variability in reporting identified across identified studies, more robust quantitative examination (i.e., meta-regression) and (multi-) collinearity diagnostics could not be undertaken as part of the current study, which may be partially accountable (due to the multiple instances of missing data) for the lack of statistical significance between some external factors and ARB/MRB occurrence in groundwater (Fig. 4). Moreover, owing to the nature of the research reporting within identified studies, it was not possible to calculate “treatment” effects (effect sizes), thus a meta-analysis could not be undertaken, as studies could not be weighted using any meaningful outcome measure. As such, retrospective pooled analyses with the analytical units (i.e. individual study findings) weighted equally was employed to identify significant trends in this research field (Figs. 4 and 5). Result suggest that studies undertaken in high income countries reported significantly lower MAR indices compared to lower and upper middle income countries ($p = 0.003$), with similar results found for MRB occurrence (i.e. lower MRB rates reported in groundwater from high income countries; $p = 0.036$). This is likely due to lower antimicrobial diversity, lack of sanitation, poor hygiene practices and reduced antimicrobial stewardship in areas characterised by lower mean incomes (Morgan et al., 2011; Ayukekbong et al., 2017); however, the asymmetry in study numbers from each area (i.e. just 22.9% in high income countries; Table 4) likely impacted study findings.

Climatologically, pooled analysis indicates that groundwater samples collected during dry periods yielded significantly higher MAR indices ($p = 0.008$) than during wet periods, directly contrasting previous findings in surface water environments (Sanderson et al., 2018). While precipitation is roundly acknowledged as a primary driver of groundwater contamination (Hynds et al., 2012; Andrade et al., 2018), in the context of antimicrobial resistance, the evidence is less compelling. For example, drier periods may enable higher concentration of antimicrobial residues within the subsurface (Dhar et al., 2008), thus leading to increased bacterial exposure to sub-therapeutic levels of antimicrobial residues. Moreover, as temperature is directly correlated with bacterial proliferation, even marginal increases in subsurface temperatures may increase bacterial loading in-situ (John and Rose, 2005). However, it is important to note that in the absence of specific hydrogeological information, interpreting the impact of seasonality on ARB and MRB occurrence is largely speculative in this instance, further reiterating the need for a more “holistic” approach to multidisciplinary groundwater research. Socio-geographically, groundwater sources located in categorically urban (as opposed to rural) regions were associated with significantly higher MAR indices ($p = 0.001$; Fig. 5). Similarly (and likely collinear with this previous finding), supplies predominantly adjacent to human waste sources (e.g. septic tanks and wastewater treatment plants) were characterised by a significantly higher ($p = 0.005$) mean MAR index than those adjacent to animal waste sources (e.g. animal grazing and manure spreading). This is possibly driven by the antimicrobial residual concentration differentials between urban and rural areas. For example, while antimicrobial concentrations in manure are typically orders of magnitude higher than those encountered in wastewaters (Arikan et al., 2009; Sabri et al., 2018), the spatiotemporal exposure is inconsistent, due to the diffuse

nature of the source. Conversely, wastewater treatment infrastructures typically result in point source contamination mechanisms, providing a consistent source of low-dose antimicrobial residues. Moreover, transmission (infiltration) of common veterinary antimicrobials in the subsurface may be confined and thus spatially limited. Transport of tetracycline compounds, in particular, are restricted to fast preferential and macropore flow or require facilitation of co-transport with mobile colloids, such as dissolved organic matter (Thiele-Bruhn, 2003). Nevertheless, an increase in the global occurrence of ARB and MRB in groundwater sources is expected due to current and future population growth, urbanisation, lack of sanitation, inappropriate wastewater treatment, and the misuse and over-use of antimicrobials, irrespective of geographic location.

Focusing on the identification of factors driving ARB and MRB occurrence, significant research challenges also exist due to inherent complexities associated with the genetic acquisition of antimicrobial resistance, in addition to the multifaceted hydrogeological mechanisms governing subsurface contamination. For example, while MAR indices calculated within Carbapenems, which are generally used as “last line” drugs (Van Boeckel et al., 2014), were found to be significantly lower than other classes, several similarly prescribed antimicrobials, such as Glycopeptides (Van Boeckel et al., 2014), were associated with relatively high mean MAR index values (Table 4). Accordingly, antimicrobial usage itself may not be a primary driver of MRB or ARB in groundwater, despite remaining an undeniably important factor in the broader antimicrobial resistance crisis. This is in line with findings from Collignon et al. (2018) that antimicrobial consumption was not significantly associated with the global indices for antimicrobial resistance.

At the genus level, there was significant variance observed in the rates of encountered antimicrobial resistance (Fig. 2; Table 5). Ubiquitous members of the soil microbiome *Pseudomonas* spp., *Klebsiella* spp. and *Enterobacter* spp. exhibited some of the highest rates of antimicrobial resistance at $99.9\% \pm 0.2$, $99.8\% \pm 0.6$ and $99.2\% \pm 2.2$, respectively. These findings are particularly significant as both *Pseudomonas* and *Klebsiella* spp. are considered to be ubiquitously “soil-resident” bacteria, and as such, may characterise the local and/or source specific resistome more accurately than faecal indicator and/or (opportunistic) pathogens, which are often absent. However, it should be noted that a proportion of this resistance is attributable to ‘intrinsic resistance’, and thus, should not be interpreted as a wholly anthropogenic phenomenon in all cases. For example, *Pseudomonas aeruginosa* is intrinsically resistant to many antimicrobial agents accredited to the low permeability of its outer membrane (Livermore, 1984), the expression of various efflux pumps with wide substrate specificity (Livermore, 2001) and the naturally occurring chromosomal AmpC β -lactamase (Nordmann and Guibert, 1998). A more compelling assessment of acquired resistance focuses on *E. coli*, which was the most frequently examined bacterial species across the studies, with pooled isolates exhibiting below average, but considerably high, rates of resistance to ≥ 1 (79.8 ± 30.1) and ≥ 3 (45.4 ± 36.0) antimicrobial agents. Mechanisms explaining between-species variation could not be determined in the current study, as it may reflect locally specific circumstances. However, drawing from previous field and modelling work (Hynds et al., 2012), findings may suggest higher rates of ARB ingress and resistance acquisition taking place in the subsurface via “traditional” recharge, with lower rates of antimicrobial resistance associated with rapid entry (i.e. runoff at wellhead and/or preferential flow) into groundwater sources. As such, it is recommended that future studies perform antimicrobial susceptibility assays, where possible, using both faecal and soil-resident bacteria to accurately establish the extent of resistance

within microbial communities in groundwater, and their most likely ingress mechanisms. Moreover, as molecular methods of antimicrobial resistance characterisation (specific gene targeting) increase in popularity, it is important that isolate-specific phenotypic (i.e. culture-based) research be undertaken to permit a greater understanding of the specific public health significance of environmental exposures to ARB."

As with any literature findings-based study, it is important to acknowledge that data used for analyses are limited to previous reports; and thus, the potential for reporting bias exists and should be highlighted (i.e. studies targeting susceptible areas or groundwater sources where ARB contamination is suspected). Over half (52.9%) of identified studies included in this review based their findings on "one-off" (i.e. non-temporal) groundwater sampling regimes, and were associated with significantly higher MAR indices ($p = 0.003$). Moreover, a widespread lack of explicit methodology for achieving sample representativeness was identified during validity appraisal (Supplementary Materials 1). As such, the occurrence rates of ARB and MRB in global groundwater garnered from this review may represent an overestimate, with true values likely more accurately reflected via means calculated from temporal studies only (i.e. ARB = $65.5\% \pm 33.4$ and MRB = 38.9 ± 32.0). However, the issue of temporal and spatial representativeness still represents a significant concern. Baseline sampling and analytical procedures should be developed and routinely employed via unbiased and robust spatiotemporal studies, following consistent methodologies, in diverse (hydro)geological and climatic settings.

The current study is the first to integrate findings from international literature regarding the occurrence of ARB and MRB in groundwater sources. While this research is undoubtedly topical, identified studies highlight a long-standing issue, with the earliest relevant research available within the scientific literature (Cooke, 1976) reporting antimicrobial resistance in 48.9% of 321 total and faecal coliform isolates (species unspecified) from groundwater sources in New Zealand. As such, and as groundwater remains an important source of potable water for a considerable proportion of the global population, the results presented show that groundwater is a significant environment where antimicrobial resistance can be spread, and highlight the need for further research looking at the occurrence of ARB and MRB in the subsurface environment.

5. Conclusion

Study results highlight groundwater as a noteworthy source of global ARB and MRB, and the pressing need for more representative studies (i.e. "baseline" work) on this topic. Additionally, lack of more robust methodologies and of key relevant data were identified as persistent issues among published work, making it difficult to ascertain a comprehensive global perspective, which is vital to quantify the risks associated with groundwater consumption and antimicrobial resistance. Thus, a key recommendation of this research is the requirement for the scientific community to refine their approach to groundwater- and health-related research. Specifically, it is imperative that future studies employ:

- hydrogeological, meteorological, climatic and detailed source-specific data measurement and reporting;
- temporal, rather than one-off, sampling methodologies;
- spatial distribution (as an attempt to achieve representativeness); and
- assessment of both faecal and soil-resident bacteria to establish the presence, origin and ingress mechanisms of antimicrobial resistant bacteria in groundwater systems.

The importance of efforts to understand and prevent the spread

of antimicrobial resistance cannot be overstated. In an era characterised by significant global challenges, including antimicrobial resistance and anthropogenic climate change, consolidation of robust research approaches and systematic design of sampling programmes to help answer pressing global questions is not only warranted, but necessary. Regardless, findings of this study offer valuable insights into the extent and significance of groundwater as a potential source of ARB, and provides guidance for future research to guide policy development, action plans and remediation efforts/technologies to safeguard public health into the future.

Author contributions

L.A. and M.K. performed the initial review of literature. All authors (L.A., M.K., P.H., J.W., A.M. and J.O.D.) determined final inclusion. Statistical analyses were carried out by L.A. in consultation with J.O.D. and P.H. All authors were involved in the writing of the manuscript, with J.O.D., P.H. and J.W. providing final approval for submission.

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.

Acknowledgements

The current study was made possible thanks to the support and funding provided by the Irish Centre for Research in Applied Geosciences (iCRAG) and Geological Survey of Ireland (GSI) under the remit of their Environmental Geosciences Postgraduate Programme.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.watres.2019.115360>.

References

- Akoachere, J.F.T.K., Omam, L.A., Massalla, T.N., 2013. Assessment of the relationship between bacteriological quality of dug-wells, hygiene behaviour and well characteristics in two cholera endemic localities in Douala, Cameroon. *BMC Public Health* 13 (1), 692.
- Andrade, L., O'Dwyer, J., O'Neill, E., Hynds, P., 2018. Surface water flooding, groundwater contamination, and enteric disease in developed countries: a scoping review of connections and consequences. *Environ. Pollut.* 236, 540–549.
- Arikan, O.A., Mulbry, W., Rice, C., 2009. Management of antibiotic residues from agricultural sources: use of composting to reduce chlortetracycline residues in beef manure from treated animals. *J. Hazard Mater.* 164 (2–3), 483–489.
- Atherholt, T.B., Procopio, N.A., Goodrow, S.M., 2017. Seasonality of coliform bacteria detection rates in New Jersey domestic wells. *Gr. Water* 55 (3), 346–361.
- Ayukekibong, J.A., Ntemgwa, M., Atabe, A.N., 2017. The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrob. Resist. Infect. Contr.* 6 (1), 47.
- Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T., Bartram, J., 2014. Fecal contamination of drinking-water in low-and middle-income countries: a systematic review and meta-analysis. *PLoS Med.* 11 (5), e1001644.
- Bell, B.G., Schellevis, F., Stobberingh, E., Goossens, H., Pringle, M., 2014. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. *BMC Infect. Dis.* 14 (1), 13.
- Bjerg, P.L., Christensen, T.H., 1992. Spatial and temporal small-scale variation in groundwater quality of a shallow sandy aquifer. *J. Hydrol.* 131 (1–4), 133–149.
- Bradford, S.A., Harvey, R.W., 2017. Future research needs involving pathogens in groundwater. *Hydrogeol. J.* 25 (4), 931–938.
- Cassini, A., Höglberg, L.D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G.S., Colomb-Cotinat, M., Kretzschmar, M.E., Devleeschauwer, B., Cecchini, M., Ouakrim, D.A., 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *Lancet*

- Infect. Dis. 19 (1), 56–66.
- Coleman, B.L., Louie, M., Salvadori, M.I., McEwen, S.A., Neumann, N., Sibley, K., Irwin, R.J., Jamieson, F.B., Daignault, D., Majury, A., Braithwaite, S., 2013. Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli*. *Water Res.* 47 (9), 3026–3036.
- Coleman, B.L., Salvadori, M.I., McGeer, A.J., Sibley, K.A., Neumann, N.F., Bondy, S.J., Gutmanis, I.A., McEwen, S.A., Lavoie, M., Strong, D., Johnson, I., 2012. The role of drinking water in the transmission of antimicrobial-resistant *E. coli*. *Epidemiol. Infect.* 140 (4), 633–642.
- Collignon, P., Beggs, J.J., Walsh, T.R., Gandra, S., Laxminarayan, R., 2018. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planet Health* 2 (9), 398–405.
- Cooke, M.D., 1976. Antibiotic resistance in coliform and faecal coliform bacteria from natural waters and effluents. *N. Z. J. Mar. Freshw. Res.* 10 (3), 391–397.
- Dhar, R.K., Zheng, Y., Stute, M., Van Geen, A., Cheng, Z., Shanewaz, M., Shamsudduha, M., Hoque, M.A., Rahman, M.W., Ahmed, K.M., 2008. Temporal variability of groundwater chemistry in shallow and deep aquifers of Arahazar, Bangladesh. *J. Contam. Hydrol.* 99 (1–4), 97–111.
- Eftim, S.E., Hong, T., Soller, J., Bom, A., Warren, I., Ichida, A., Nappier, S.P., 2017. Occurrence of norovirus in raw sewage - a systematic literature review and meta-analysis. *Water Res.* 111, 366e374.
- European Commission, 2011. Communication from the commission to the European parliament and the council: action plan against the rising threats from antimicrobial resistance. Available at: http://ec.europa.eu/dgs/health_food-safety/docs/communication_amr_2011_748_en.pdf.
- Hynds, P.D., Misstear, B.D., Gill, L.W., 2012. Development of a microbial contamination susceptibility model for private domestic groundwater sources. *Water Resour. Res.* 48 (12).
- Hynds, P.D., Thomas, M.K., Pintar, K.D.M., 2014. Contamination of groundwater systems in the US and Canada by enteric pathogens, 1990–2013: a review and pooled-analysis. *PLoS One* 9 (5), e93301.
- Interagency Coordination Group, 2019. No Time to Wait: securing the future from drug-resistant infections. Available at: <https://www.who.int/antimicrobial-resistance/interagency-coordination-group/final-report/en/>.
- John, D.E., Rose, J.B., 2005. Review of factors affecting microbial survival in groundwater. *Environ. Sci. Technol.* 39 (19), 7345–7356.
- Krumpal, P.H., 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Environ. Microbiol.* 46 (1), 165–170.
- Larsson, D.J., Andremont, A., Bengtsson-Palme, J., Brandt, K.K., de Roda Husman, A.M., Fagerstedt, P., Fick, J., Flach, C.F., Gaze, W.H., Kuroda, M., Kvint, K., 2018. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. *Environ. Int.* 117, 132–138.
- Laxminarayan, R., Duse, A., Watal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., Greko, C., 2013. Antibiotic resistance—the need for global solutions. *Lancet Infect. Dis.* 13 (12), 1057–1098.
- Livermore, D.M., 1984. Penicillin-binding proteins, porins and outer-membrane permeability of carbenicillin-resistant and -susceptible strains of *Pseudomonas aeruginosa*. *J. Med. Microbiol.* 18, 261–270.
- Livermore, D.M., 2001. Of *Pseudomonas*, porins, pumps and carbapenems. *J. Antimicrob. Chemother.* 47, 247–250.
- Maran, N., Crispim, B., Iahnn, S., Araújo, R., Grisolia, A., Oliveira, K., 2016. Depth and well type related to groundwater microbiological contamination. *Int. J. Environ. Res. Public Health* 13 (10), 1036.
- Morgan, D.J., Okeke, I.N., Laxminarayan, R., Perencevich, E.N., Weisenberg, S., 2011. Non-prescription antimicrobial use worldwide: a systematic review. *Lancet Infect. Dis.* 11 (9), 692–701.
- Morvan, X., Mouvet, C., Baran, N., Gutierrez, A., 2006. Pesticides in the groundwater of a spring draining a sandy aquifer: temporal variability of concentrations and fluxes. *J. Contam. Hydrol.* 87 (3–4), 176–190.
- Munita, J.M., Arias, C.A., 2016. Mechanisms of antibiotic resistance. *Microbiol. Spectr.* 4 (2).
- Murphy, H.M., Prioleau, M.D., Borchardt, M.A., Hynds, P.D., 2017. Epidemiological evidence of groundwater contribution to global enteric disease, 1948–2015. *Hydrogeol. J.* 25 (4), 981–1001.
- Nappier, S.P., Hong, T., Ichida, A., Goldstone, A., Eftim, S.E., 2019. Occurrence of coliphage in raw wastewater and in ambient water: a meta-analysis. *Water Res.* 153, 263–273.
- Nordmann, P., Guibert, M., 1998. Extended-spectrum β -lactamases in *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* 42, 128–131.
- O'Dwyer, J., Hynds, P., Pot, M., Adley, C.C., Ryan, M.P., 2017. Evaluation of levels of antibiotic resistance in groundwater-derived *E. coli* isolates in the Midwest of Ireland and elucidation of potential predictors of resistance. *Hydrogeol. J.* 25 (4), 939–951.
- O'Neill, J., 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations. HM Government and Wellcome Trust, UK.
- O'Flaherty, E., Cummins, E., 2017. Antibiotic resistance in surface water ecosystems: presence in the aquatic environment, prevention strategies, and risk assessment. *Hum. Ecol. Risk Assess. Int. J.* 23 (2), 299–322.
- Opatowski, M., Tuppin, P., Cosker, K., Touat, M., De Lagasnerie, G., Guillemot, D., Salomon, J., Brun-Buisson, C., Watier, L., 2019. Hospitalisations with infections related to antimicrobial-resistant bacteria from the French nationwide hospital discharge database, 2016. *Epidemiol. Infect.* 147.
- Pacheco Castro, R., Pacheco Ávila, J., Ye, M., Cabrera Sansores, A., 2018. Groundwater quality: analysis of its temporal and spatial variability in a karst aquifer. *Gr. Water* 56 (1), 62–72.
- Peel, M.C., Finlayson, B.L., McMahon, T.A., 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci. Discuss.* 4 (2), 439–473.
- Pullin, A., Frampton, G., Livoreil, B., Petrokofsky, G., 2018. Guidelines and Standards for Evidence Synthesis in Environmental Management. Collaboration for Environmental Evidence.
- Reinthal, F.F., Posch, J., Feierl, G., Wüst, G., Haas, D., Ruckebauer, G., Mascher, F., Marth, E., 2003. Antibiotic resistance of *E. coli* in sewage and sludge. *Water Res.* 37 (8), 1685–1690.
- Ribeiro, A.F., Bodilis, J., Alonso, L., Buquet, S., Feuilloley, M., Dupont, J.P., Pawlak, B., 2014. Occurrence of multi-antibiotic resistant *Pseudomonas* spp. in drinking water produced from karstic hydrosystems. *Sci. Total Environ.* 490, 370–378.
- Rodloff, A., Bauer, T., Ewig, S., Kujath, P., Müller, E., 2008. Susceptible, intermediate, and resistant—the intensity of antibiotic action. *Dtsch. Ärzteblatt Int.* 105 (39), 657.
- Sabri, N.A., Schmitt, H., Van der Zaan, B., Gerritsen, H.W., Zuidema, T., Rijnaarts, H.H.M., Langenhoff, A.A.M., 2018. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in The Netherlands. *J. Environ. Chem. Eng.* In press.
- Sanderson, C.E., Fox, J.T., Dougherty, E.R., Cameron, A., Alexander, K.A., 2018. The changing face of water: a dynamic reflection of antibiotic resistance across landscapes. *Front. Microbiol.* 9, 1894.
- Schets, F.M., During, M., Italiaander, R., Heijnen, L., Rutjes, S.A., Van der Zwaluw, W.K., de Roda Husman, A.M., 2005. *Escherichia coli* O157: H7 in drinking water from private water supplies in The Netherlands. *Water Res.* 39 (18), 4485–4493.
- Suzuki, S., Pruden, A., Virta, M., Zhang, T., 2017. Antibiotic resistance in aquatic systems. *Front. Microbiol.* 8, 14.
- Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils—a review. *J. Plant Nutr. Soil Sci.* 166 (2), 145–167.
- Traoré, O., Nyholm, O., Siitonen, A., Bonkoungou, I.J.O., Traoré, A.S., Barro, N., Haukka, K., 2015. Prevalence and diversity of *Salmonella enterica* in water, fish and lettuce in Ouagadougou, Burkina Faso. *BMC Microbiol.* 15 (1), 151.
- Van Boeckel, T.P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., Laxminarayan, R., 2014. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* 14 (8), 742–750.
- Van Schaik, W., 2015. The human gut resistome. *Philos. Trans. R. Soc. Biol. Sci.* 370 (1670), 20140087.
- Walczak, J.J., Bardy, S.L., Feriancikova, L., Xu, S., 2011. Influence of tetracycline resistance on the transport of manure-derived *Escherichia coli* in saturated porous media. *Water Res.* 45 (4), 1681–1690.
- Wallender, E.K., Ailes, E.C., Yoder, J.S., Roberts, V.A., Brunkard, J.M., 2014. Contributing factors to disease outbreaks associated with untreated groundwater. *Gr. Water* 52 (6), 886–897.
- Wellington, E.M., Boxall, A.B., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M., Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., Thomas, C.M., 2013. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect. Dis.* 13 (2), 155–165.
- Williams-Nguyen, J., Sallach, J.B., Bartelt-Hunt, S., Boxall, A.B., Durso, L.M., McLain, J.E., Singer, R.S., Snow, D.D., Zilles, J.L., 2016. Antibiotics and antibiotic resistance in agroecosystems: state of the science. *J. Environ. Qual.* 45 (2), 394–406.
- World Bank, 2018. Country and lending groups. Available at: <https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups>. (Accessed 5 March 2018).
- World Health Organisation, 2015. Global action plan on antimicrobial resistance. Available at: http://apps.who.int/iris/bitstream/10665/193736/1/9789241509763_eng.pdf?ua=1.
- World Health Organisation, 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Available at: <https://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/>.
- World Health Organisation, 2018. Antimicrobial resistance. Available at: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>.
- Wright, G.D., 2010. Antibiotic resistance in the environment: a link to the clinic? *Curr. Opin. Microbiol.* 13 (5), 589–594.