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Monitoring of Antimicrobials in Australia: Prioritisation and Analytical Approaches for Environments and Integrated Systems

SAAFE[CRC]

Authors:

Emily May Stevenson; Jake O'Brien; Kevin Thomas; Monique Binet; Nick Ashbolt; Claire Hayward; Greg Jackson; Carly Beggs

Reviewers:

Mark Drew; Andrew Barnes; Matt Landos; Paul Monis; SAAFE[CRC] (Mary Carr); Vitibit Pty Ltd (Liz Riley); and anonymous reviewers from the water and horticulture industries.

Research Contractor:

Queensland Alliance for Environmental Health Sciences, The University of Queensland

SAAFE Project Manager:

Erica Donner; Kelly Hill

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This document is under development and intended as general guidance material for a wide range of sectors. This report should be treated as a 'live document', and we extend an open invitation for any feedback or revisions necessary. If you would like to make any suggestions, please contact SAAFE[CRC]: enquiries@crcsaafe.com.au.

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Acknowledgment of Country

SAAFE and partners acknowledge the Traditional Custodians of the lands, seas and waters of this Country, and of all areas in which we live and work across Australia.

We respect Aboriginal and Torres Strait Islander Peoples' deep cultural and spiritual relationship with this land and pay our respects to Elders past and present.

We acknowledge the diversity of Aboriginal peoples and their knowledge systems. We recognise the concept of One Health (which highlights the integrated nature of human, animal, plant and environmental health) is not something new to Aboriginal peoples, and that embracing this knowledge and connection to Country is a vital step in the path to reconciliation.

SAAFE is respectfully committed to a research program underpinned by the core value of Caring for Country.

Executive Summary

Monitoring of antimicrobials and their transformation products presents a strategic opportunity for Australia to strengthen environmental stewardship and demonstrate global leadership in One Health implementation.

Antimicrobials are defined as chemicals and their relevant metabolites/transformation products that have antimicrobial properties. We refer to these collectively as 'antimicrobials'. Protecting their long-term effectiveness requires responsible use and ongoing efforts to understand and manage antimicrobial resistance (AMR) across One Health settings.

In alignment with the Australian National AMR Strategy and related Federal programs spanning environment, agriculture, biosecurity and public health, coordinated environmental antimicrobial monitoring provides a practical pathway to provide local evidence to support risk-based decision-making. Strengthening national monitoring capability also positions Australia to demonstrate high standards of antimicrobial stewardship.

As environmental antimicrobial monitoring continues to evolve in Australia, there is an opportunity to strengthen national capability through harmonisation and prioritisation. This report contributes to that effort through the following aims:

1. Produce a **customised** and **prioritised** list of **antimicrobials** (analytes) of potential concern for both **aquatic** and **agribusiness** environmental settings.

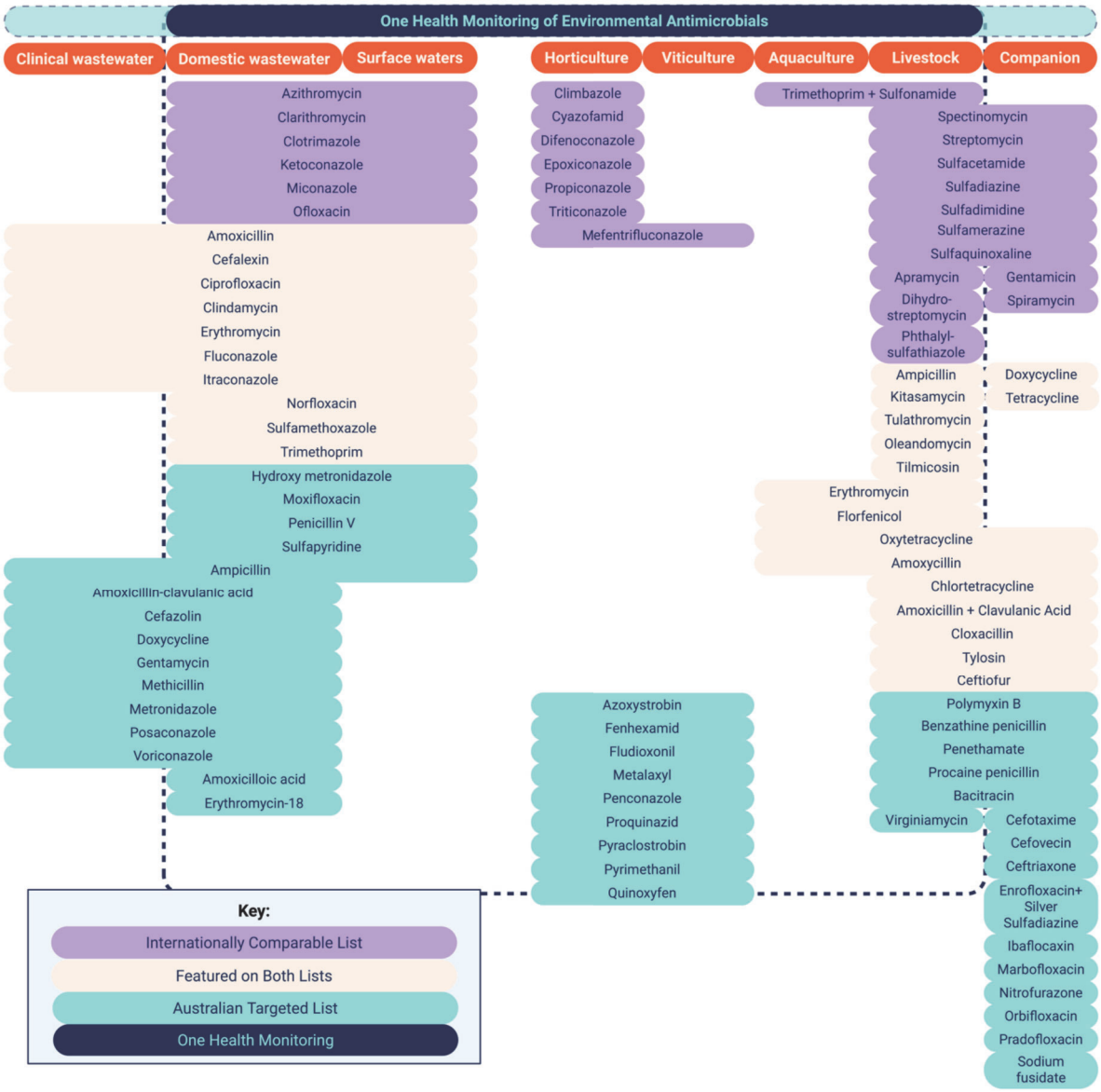
2. Present a **decision-making framework** for analytical techniques for **antimicrobial identification** and/or **quantification** in waters and agribusiness environments.

3. Describe **core analytical detection methods** currently available for antimicrobials in waters and agribusiness environments, including their strengths and weaknesses and recommendations for applications.

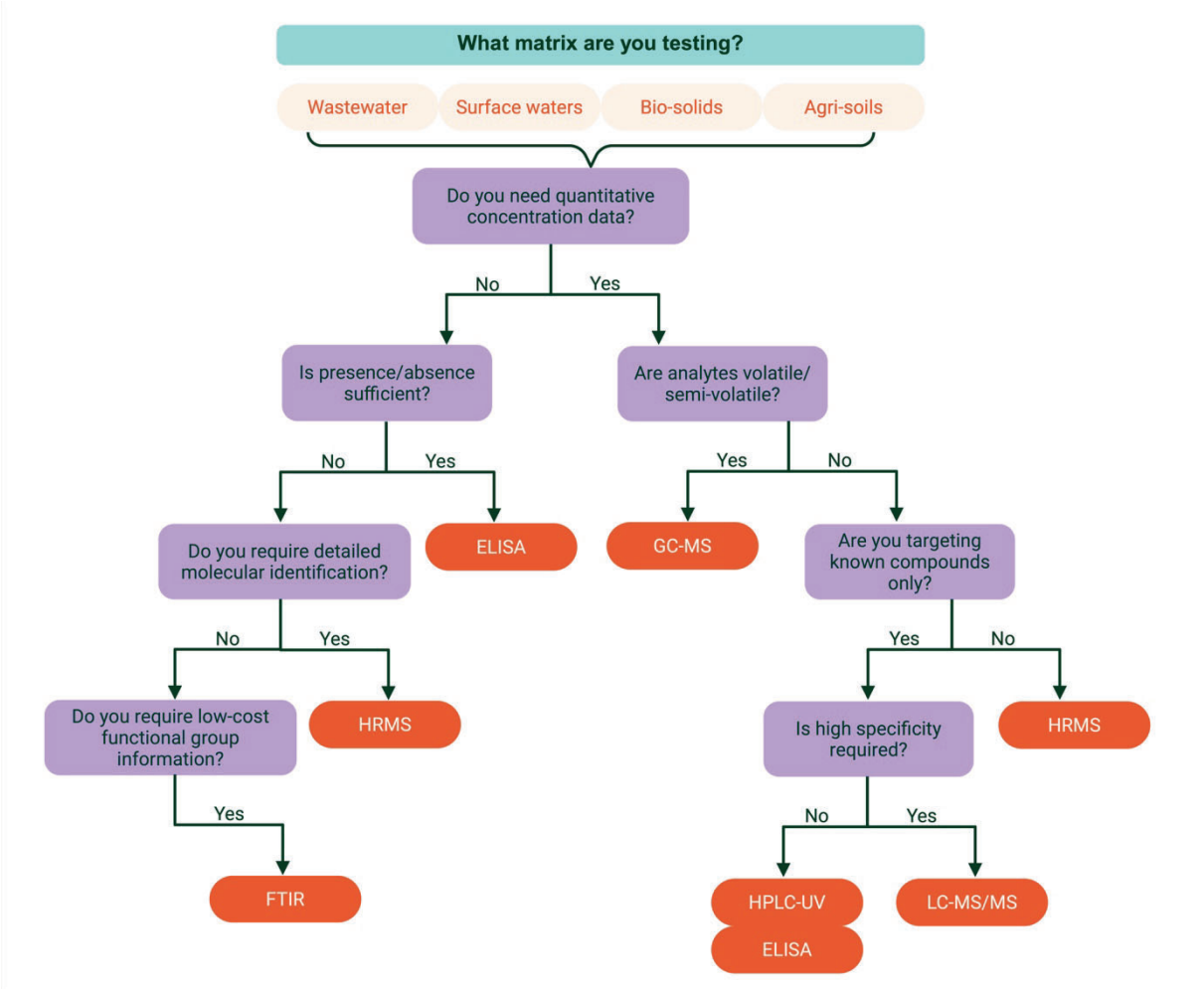
4. Identify **opportunities** and **priorities** for advancing **antimicrobial monitoring** tools to support future AMR-related research, monitoring and practical applications.

We first propose a framework for selecting priority antimicrobials for monitoring across aquatic and agribusiness environments in Australia, aligned with the objectives of key stakeholders. For each setting, two priority lists are defined: (1) antimicrobials of global importance to support international benchmarking, and (2) Australia-specific priorities, capturing compounds of national relevance.

Given the interconnected nature of antimicrobial pathways across One Health settings, we also present a schematic to enable rapid prioritisation and visualisation of antimicrobials of both international and national relevance, per industry. While this framework supports pragmatic decision-making where resources need to be prioritised, the most comprehensive approach – where time and resources permit – would be to monitor all compounds which fall under the One Health monitoring framework:



Alongside identifying priority analytes, we present a simplified decision-making framework to support the selection of analytical techniques, based on matrix type, analytical requirements, study objectives, and target compound properties:



By bringing together priority antimicrobials, technical monitoring tools and sector-specific (aquatic or agribusiness) applications, this report lays the foundations for a more cohesive and fit-for-purpose One Health approach to monitoring the occurrence of antimicrobials in Australian environments. In doing so, it supports delivery of national AMR objectives, strengthens the evidence available to inform policy and regulation, and contributes to Australia's ability to demonstrate high standards of environmental protection, biosecurity and antimicrobial stewardship against international benchmarks.

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Abbreviations

AMR	Antimicrobial Resistance
ANZG	Australian and New Zealand Guidelines for Fresh and Marine Water Quality
API	Active pharmaceutical ingredient
APVMA	Australian Pesticides and Veterinary Medicines Authority
ASTAG	Australian Strategic and Technical Advisory Group on AMR
AURA	Antimicrobial Use and Resistance in Australia
AWRI	Australian Wine Research Institute
DAFF	Department of Agriculture, Fisheries and Forestry
DDD	Defined daily doses
DGV	Default guideline value
ELISA	Enzyme-linked Immunosorbent Assays
EPA	Environmental Protection Agency
EU	European Union
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
GVs	Guideline values
HPLC-UV	High-performance Liquid Chromatography with Ultraviolet Detection
HRMS	High-Resolution Mass Spectrometry
LC-MS/MS or LC-MS2	Liquid Chromatography-Tandem Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MEC	Measured environmental concentration
MIC	Minimum inhibitory concentrations
MRL	Maximum residue limit
MRSA	Methicillin resistant *Staphylococcus aureus*
PNEC	Predicted no effect concentration
PNECR	Predicted no effect concentration for resistance
Q-TOF	Quadrupole-Time-of-Flight
QA	Quality assurance
QLD	Queensland
QQQ	Triple Quadrupole
SAAFE	Solving Antimicrobial Resistance in Agribusiness, Food & Environments
SPE	Solid-phase extraction
TGA	Therapeutic Goods Administration
WHO	World Health Organisation
WOAH	World Organisation for Animal Health
WWTP	Wastewater treatment plant

1. Introduction

Antimicrobials are defined as chemicals and their relevant metabolites/transformation products that have antimicrobial properties. There are several key types of antimicrobials, each designed to treat specific groups of microorganisms. Antibacterials or antibiotics (herein referred to as antibacterials) are used to treat bacteria, antifungals target fungi, antivirals target viruses, and antiparasitic agents target parasitic microorganisms. We refer to these collectively as antimicrobials.

Since the discovery of penicillin, antimicrobial compounds have become a cornerstone of modern medicine. In 2018, global defined daily doses (DDD) of antibacterials alone were estimated to be 40 billion [1,2]. Beyond human healthcare, antimicrobials are also widely used in animal and plant health, agricultural practices, food preservation and in the wider agribusiness industry, together account for approximately 70% of global antimicrobials used [3].

Australia's healthcare and agricultural sectors rely on antimicrobials to support human, animal and plant health. Australia has invested in longstanding national surveillance systems for antimicrobial use and resistance that provide an important foundation for antimicrobial stewardship, treatment guidelines and evidence-based decision-making. The latest AURA Report (2026) states that antimicrobial use remains below 2015 levels, emphasising the value of Australia's stewardship and surveillance programs [4].

Extending this capability into environmental antimicrobial monitoring represents a natural progression of Australia's One Health approach to AMR. Australia's extensive and diverse agricultural industries across all states and territories further highlight the importance of coordinated environmental monitoring to support evidence-based stewardship, maintain Australia's high standards, and demonstrate leadership in One Health antimicrobial management.

1.1. Environmental pathways of antimicrobials

There are several pathways for antimicrobials to enter the environment [5,6]. Within human health, antimicrobials can be ingested, topically applied or injected, and most active pharmaceutical ingredients (APIs) – biologically active components of pharmaceuticals including metabolites and transformation products [7] – are not fully metabolised and excreted within urine or faeces, and make their way to wastewater treatment plants (WWTPs) or onsite treatment facilities such as septic tanks.

Within WWTPs, treatment processes reduce but do not completely remove all antimicrobial compounds. As a result, some antimicrobials and their transformation products may remain detectable in treated wastewater, aquatic environments via wastewater effluent, septic tank discharge, or sewer overflows. Under some environmental conditions, residual concentrations may contribute to selection pressures associated with antimicrobial resistance (AMR) [8].

APIs may also enter the environment through a range of pathways, including industrial effluents and the reuse of biosolids. From these pathways, antimicrobial compounds may be transported to surrounding environments through processes such as runoff, drainage and infiltration[10].

Similarly, in animal or food-production systems (inclusive of agriculture and aquaculture), antimicrobials are not fully metabolised within animal bodies, where even ‘complete degradation’ may result in the emission of transformation products or metabolites. Plant protection products (e.g. herbicides, insecticides, fungicides) may also incorporate antimicrobial compounds, meaning agricultural soils are another possible environmental pathway[9]. Whilst there are data available on global antimicrobial concentrations in agricultural systems [10–12], comparable datasets

remain relatively limited in Australia, highlighting the value of environmental monitoring to better understand the Australian landscapes.

1.2. Introduction to One Health

The One Health concept recognises that human, animal, plant and environmental health are inherently interconnected. The concept of One Health is integral to understanding the occurrence and management of antimicrobials in the environment, in that all aspects of this threat represent a true One Health challenge (Figure 1). In the eyes of the World Health Organisation's (WHO) One Health High-Level Expert Panel [13]:

"One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems"

Figure 1. *A 'One Health' approach to managing AMR recognises that populations, species, facilities, and industries co-exist within environments, and are connected by water/air and the movement of people, animals, plants, goods and materials. The drivers of AMR, as well as the resistant organisms and their genes (all represented here by white dots), may likewise be connected across populations, species and environments. Image © SAAFE [14].*

1.3. Antimicrobial Resistance

Antimicrobial resistance or AMR describes the ability of microorganisms to survive or continue growing in the presence of an antimicrobial agent. As resistance develops, some infections and diseases in humans, animals and plants can become more difficult to manage, creating implications for healthcare, veterinary medicine, agriculture and food production systems. Recognising these broad societal and economic implications, the World Health Organization (WHO) has identified AMR as a major global health challenge[15].

Some microorganisms are naturally, or inherently, resistant to specific antimicrobials. Resistance may also evolve or be acquired in response to selection pressures. Selection can be direct, leading to the emergence of a resistance gene or AMR determinant specific to the antimicrobial

to which a bacterium is exposed, although, other co-selective pressures do exist. These include, but are not limited to, exposure to organic and inorganic chemicals (both individually and as mixtures) [16,17]. AMR evolution can result from genetic mutations, recombination, or horizontal gene transfer, which involves the transfer of genetic material between vectors or pathogens (bacterial cells) [18].

1.4. Australia's National Antimicrobial Resistance Strategy

Australia has maintained a coordinated national approach to AMR for more than a decade through successive National AMR Strategies. These strategies are underpinned by a One Health framework, recognising the interconnected nature of human, animal, plant and environmental health.

A central theme across both the 2015–2020 Strategy [19] and the current *Australia's National Antimicrobial Resistance Strategy – 2020 and Beyond* [20] is the importance of surveillance, monitoring and evidence generation to support informed decision-making, stewardship and policy development.

Within this context, chemical monitoring of environmental antimicrobials provides an important complementary evidence stream. Monitoring can support a better understanding of the occurrence, distribution and fate of antimicrobial compounds across environmental, agricultural and food production systems, helping to inform management approaches and contribute to broader One Health surveillance objectives.

This report has been developed within that policy context, providing an overview of available monitoring approaches, analytical tools and implementation considerations relevant to antimicrobial residue monitoring across Australia.

1.5. Aims of this report

This report lays the foundation for applying currently available analytical techniques to strengthen antimicrobial monitoring and support the development of new techniques. This will be addressed through four sub-objectives:

1. Produce a customised and prioritised list of antimicrobials (analytes) of potential concern for both aquatic and agribusiness environmental settings.

2. Present a Decision-Making Framework for analytical techniques for antimicrobial identification and/or quantification in waters and agribusiness environments.

3. Describe core analytical detection methods currently available for antimicrobials in waters and agribusiness environments, including their strengths and weaknesses and recommendations for applications.

4. Identify opportunities and priorities for advancing antimicrobial monitoring tools to support future AMR-related research, monitoring and practical applications.

2. Identifying Priority Antimicrobials

Antimicrobial resistance may evolve under a direct selection pressure – where a specific compound selects for resistance to that same compound – or through an indirect selection pressure. Indirect selection for AMR is called co-selection, and several different types of micropollutants have been shown to contribute to co-selection for AMR [16], including: metals & metalloids [21], biocides [22–25], fungicides [26], microplastics [17,27], plastic additives [28] and non-antibacterial drugs [29].

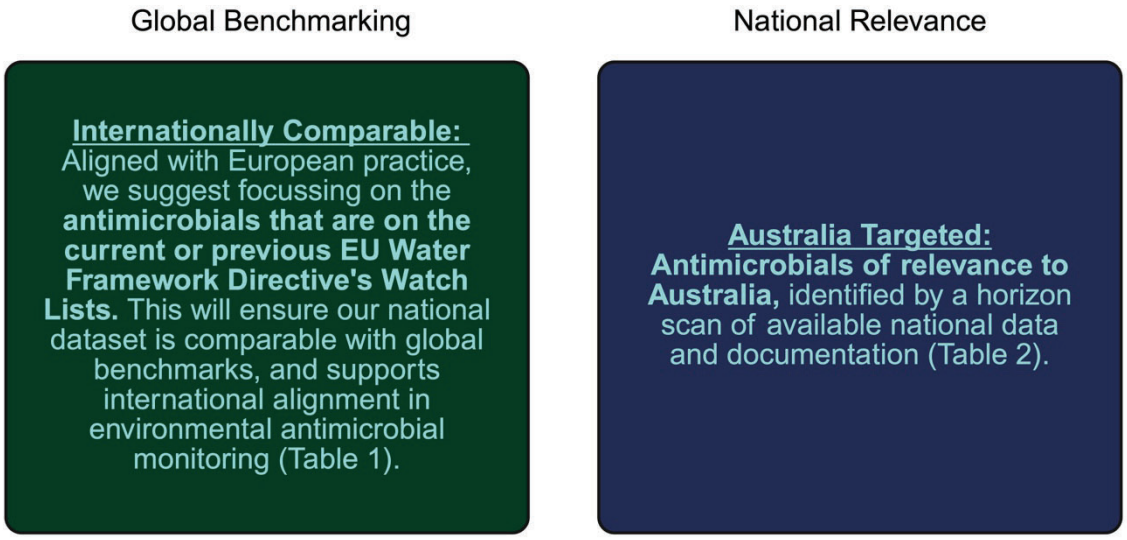
The relative contribution of antimicrobials versus other compounds (e.g. metals, non-antimicrobial pharmaceuticals and various pesticides), however, remains unclear. **Accordingly, this report focuses on existing evidence of antimicrobial parent compound concentrations across Australia, and where possible, their transformation products.**

Furthermore, whilst a wide range of different compounds are used and detected in environmental settings, it is important to consider financial and time constraints when designing monitoring programs. Therefore, we have applied a strategy-based approach to prioritising **antimicrobial compounds for monitoring** in Australian aquatic (2.1) and agribusiness environments (2.2).

The strategy-based approach is designed to support practical prioritisation, not exclusion. Where time, budget and technical capability permit, monitoring antimicrobials across both priority strategies provides the most comprehensive assessment of environmental occurrence.

2.1. Priority antimicrobials in aquatic environments

Aquatic environments (wastewater, surface waters etc.) receive antimicrobial inputs from clinical, domestic and, in some cases, industrial, veterinary and agricultural sources. Given the diversity of inputs, numerous antimicrobial analytes may be relevant for monitoring. For the prioritisation of antimicrobials in aquatic environments, a strategy-based system is proposed which considers two key stakeholder strategies: international benchmarking and national relevance for Australia:



This approach considers:

- Time and budget constraints which influence the number of analytes that can be measured.
- Availability of analytical instrumentation, standards, and methods, which may further influence the number of analytes that can be included.
- Including antimicrobials from current EU Water Framework Directive Watch Lists to support international benchmarking and comparison with established monitoring programs.
- Retaining antimicrobials included in earlier Watch Lists which enables Australian data generation for compounds that have completed their data collection cycle in the EU.
- Capturing Australian-specific substances to ensure that national priorities are reflected in ongoing and future monitoring efforts.

To ensure relevance to Australia, all compounds were searched in the Therapeutic Goods Administration's (TGA) 'Australian Register of Therapeutic Goods' (ARTG) Search Visualisation Tool and database to confirm their registration or approval for use in Australia before inclusion in either list. **All compounds not registered for use in Australia with the TGA were excluded from the prioritisation framework.**

Strategy 1 (Table1) is recommended as the core suite of analytes for aquatic environmental monitoring where international benchmarking is an objective. By aligning with the current and previous EU Water Framework Directive Watch Lists, Strategy 1 supports international benchmarking while providing a practical and harmonised foundation for environmental antimicrobial monitoring in Australia.

The EU Water Framework Directive Watch List provides a coordinated framework for prioritising and monitoring emerging compounds across Europe. By harmonising monitoring across European Member States, it has established a valuable evidence base for priority compounds and enables direct comparison of monitoring data between jurisdictions. Compounds included on the Watch List, are monitored by EU Member states over a defined monitoring cycle, generating consistent datasets that support future prioritisation, analytical method development and scientific understanding of environmental occurrence. The current Watch List was published in

2025 and includes 4 priority antibacterials and 10 priority antifungals [30]. Three of these compounds are registered on the TGA's ARTG Search and Visualisation Tool [31], and were therefore included in Strategy 1 (Table 1). Monitoring these antimicrobials in Australia will enable direct comparison with international datasets while contributing Australian evidence to the global knowledge base for priority antimicrobial compounds.

Although bromuconazole is listed on the current Watch List, it is neither registered with the TGA nor in the APVMA's (Australian Pesticides and Veterinary Medicines Authority) search tool - PubCRIS [32]. Therefore, this compound has not been included within the Australian aquatic prioritisation framework.

The remaining compounds in the current Watch List were registered only with the APVMA, not the TGA, and have therefore been incorporated into the agribusiness prioritisation framework (Section 2.2).

Strategy 1 also includes all antimicrobials that are listed on previous EU Watch Lists (2022 [33], 2020 [34], 2018 [35] and 2015 [36]). With each new iteration of the Watch List, compounds are only retained for a defined monitoring cycle of up to 4 years, during which EU Member States generate monitoring data. Including compounds from previous Watch Lists enables Australia to generate comparable datasets for analytes that have completed their European monitoring cycle, while maintaining alignment with internationally recognised monitoring priorities for environmental antimicrobial monitoring.

For aquatic stakeholders seeking greater emphasis on Australian priorities, Strategy 2 (Table 2) expands the framework to include analytes of particular interest within Australian aquatic environments. This list is informed by a horizon scan of nationally relevant sources, including:

- Australian Commission on Safety and Quality in Health Care (2023). AURA 2023: Fifth Australian report on antimicrobial use and resistance in human health. Sydney: ACSQHC.
- Australian Commission on Safety and Quality in Health Care (2026). AURA: Sixth Australian report on antimicrobial use and resistance in human health. Sydney: ACSQHC.
- Li, J., O'Brien, J.W., Tscharke, B.J., He, C., Shimko, K.M., Shao, X., Zhai, N., Mueller, J.F. and Thomas, K.V., 2024. *National survey of the occurrence of antimicrobial agents in Australian wastewater and their socioeconomic correlates*. Nature Water, 2(12), pp.1166-1177.
- Li, J., O'Brien, J.W., Tscharke, B.J., Verhagen, R., He, C., Shimko, K.M., Shao, X., Zhai, N., Hulleman, T., Mueller, J.F. and Thomas, K.V., 2025. *Occurrence, removal, and risk assessment of antimicrobials and their transformation products in effluent from Australian wastewater treatment plants*. Environmental Science & Technology, 59(13), pp.6825-6838.

Table 2 should not be interpreted as a systematic or exhaustive list. Rather, it provides a practical baseline that can be refined as additional Australian monitoring and evidence becomes available.

Table 1. International benchmarking priority compounds *(international comprehensive)* ***for aquatic monitoring in Australia,*** *adopted from current and previous EU Watch Lists. Only antimicrobials registered for use with the Australian Government's Department of Health and Aged Care Therapeutic Goods Administration ARTG Search Visualisation Tool [31]. **Emboldened text** highlights compounds included in our national relevance tier below.*

Compound	Type	Class	Persistent (P) Bioaccumulative (B), Toxic (T)	EU Watch List
Norfloxacin	Antibacterial	Fluroquinolone	Does not currently meet PBT criteria [30]	2025
Erythromycin		Macrolide	P (suspected), T [37]	2015
Clarithromycin		Macrolide	P (suspected), T [38]	2015
Azithromycin		Macrolide	P, T [39]	2015
Amoxicillin		Penicillin	P (suspected), T [40]	2018
Ciprofloxacin		Fluoroquinolone	P (suspected), T [41]	2018
Sulfamethoxazole		Sulfonamide	P, T (suspected) [34]	2020
Trimethoprim		Diaminopyrimidine	P, T (suspected) [34]	2020
Cefalexin		Cephalosporin	No data	2022
Clindamycin		Lincosamide	P (suspected), T (suspected) [42]	2022
Ofloxacin		Fluoroquinolone	No data	2022
Itraconazole	Antifungal	Azole (triazole)	T [30]	2025
Ketoconazole		Azole (imidazole)	P (suspected), B, T [30]	2025
Clotrimazole		Azole (imidazole)	P, B (suspected) [34]	2020
Fluconazole		Azole (triazole)	No data	2020
Miconazole		Azole (imidazole)	P, B, T (suspected) [34]	2020

***Table 2. Australia-specific priority antimicrobial compounds (AU targeted) for aquatic monitoring in Australia**, identified by horizon scan. **Emboldened text** highlights compounds currently or previously listed on a WFD Watch List.*

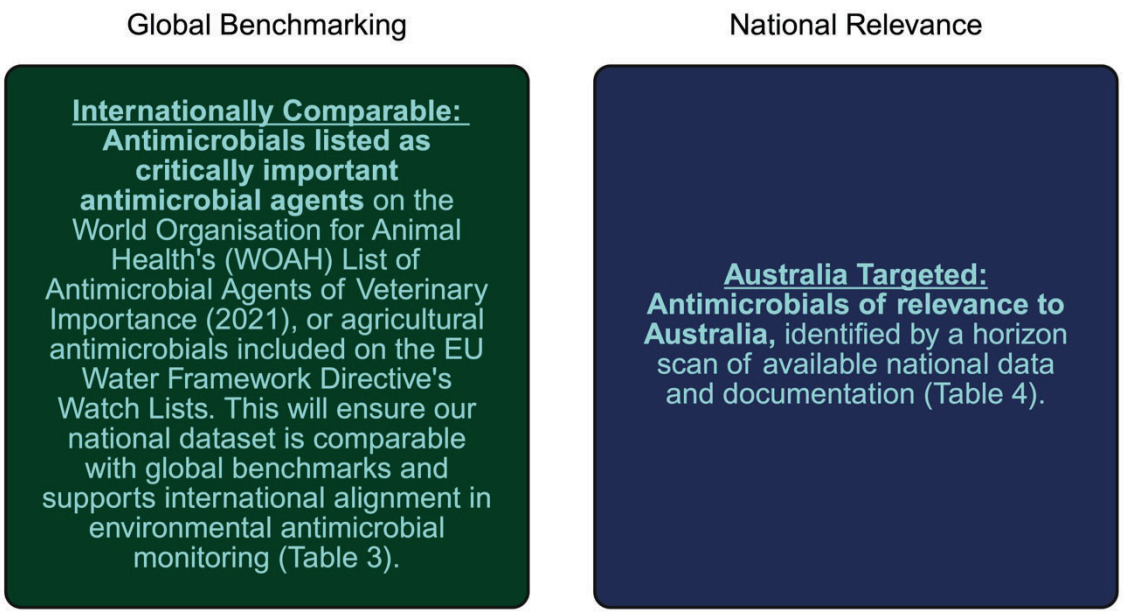
Antimicrobial	Type	Class	Industry Priority	Reason	ref
Amoxicillin	Antibacterial	Penicillin	Clinical wastewater; Domestic wastewater	Amongst most used oral antibacterials in Australian acute care hospitals. Amongst top 4 most frequently dispensed antimicrobial in the community in 2024.	[43,4]
Cefalexin		Cephalosporin		Amongst most used oral antibacterials in Australian acute care hospitals. Amongst top 4 most frequently dispensed antimicrobial in the community in 2024. Occurred at the highest concentrations across Australian WWTPs and population-normalised mass loads in Li *et al.* [44]	
Cefazolin				Amongst most used antimicrobial by volume in Australian operating theatres.	
Doxycycline		Tetracycline		Amongst most used oral antibacterials in Australian acute care hospitals. Amongst top 4 most frequently dispensed antimicrobial in the community in 2024.	
Erythromycin		Macrolide		High levels of resistance (35% detected in Australian *Streptococcus* strains.	
Gentamycin				Amongst most used antimicrobial by volume in Australian operating theatres.	
Methicillin		Beta-lactam		High prevalence of community-acquired methicillin resistant *Staphylococcus aureus* (MRSA) clones.	
Amoxicillin-clavulanic acid	Antibacterial combination	Beta-lactam with beta-lactamase inhibitor		Amongst most used oral antibacterials in Australian acute care hospitals. Amongst top 4 most frequently dispensed antimicrobial in the community in 2024.	
Itraconazole	Antifungal	Azole (triazole)		Amongst most used antifungals across Australia.	
Posaconazole					
Voriconazole					
Ampicillin	Antibacterial	Penicillin	Clinical wastewater;	Found at concentrations predicted to pose medium selection risk at 6 WWTPs across Australia; more than half of the measured concentrations surpassed $PNEC_R$;	[4,43–45]

			Domestic wastewater; Surface waters	common resistance in *Escherichia coli* (*E. coli*) and is intrinsically resistant in *Klebsiella pneumoniae* and *Enterobacter cloacae* complexes.	
Ciprofloxacin		Fluoroquinolone		Consistently found at concentrations predicted to pose medium-high selection risk across Australian WWTP effluent; detected across all 50 sites showing national, widespread usage; all measured WWTP influent concentrations exceeding $PNEC_R$; over 74% of notified Australian *Salmonella* strains expressed ciprofloxacin resistance.	
Fluconazole	Antifungal	Azole (triazole)		Amongst most used antifungals across Australia; detected across all 50 sites showing national, widespread usage; found to pose medium risk for AMR selection at several WWTP sites across Australia. Most prescribed antifungal in acute hospital settings in 2022 and 2023.	
Clindamycin	Antibacterial	Lincosamide	Clinical wastewater; Domestic wastewater	Detected across all 50 sites showing national, widespread usage; 35% of notified, Australian *Streptococcus* strains expressed clindamycin resistance 35%.	[43,44]
Metronidazole		Nitroimidazole		Amongst most used antimicrobial by volume in Australian operating theatres; detected across all 50 sites showing national, widespread usage.	
Moxifloxacin		Fluroquinolone	Domestic wastewater; Surface waters	Some measured WWTP influent concentrations exceeded $PNEC_R$.	[44]
Norfloxacin				Some measured WWTP influent concentrations exceeded $PNEC_R$.	
Sulfa-methoxazole		Sulfonamide		Detected across all 50 sites showing national, widespread usage; over 50% of samples exceeded $PNEC_R$.	
Sulfapyridine				Detected across all 50 sites showing national, widespread usage; over 50% of samples exceeded $PNEC_R$.	

Trimethoprim		Diaminopyrimidine	Domestic wastewater	Detected across all 50 sites showing national, widespread usage.	
Amoxicilloic acid	Trans-formation product	Penicillin (metabolite)		Occurred at the highest concentrations and population-normalised mass loads.	
Erythromycin-18		Macrolide (metabolite)		Detected across all 50 sites showing national, widespread usage.	
Hydroxy metronidazole		Nitroimidazole (metabolite)	Domestic wastewater; Surface waters	Ten times more potent in triggering gene mutations than parent compound (metronidazole), and yet detected at higher concentrations; metronidazole reached $PNEC_R$ in half of the influent samples collected.	[44,46]
Penicillin V	Antibacterial	Penicillin (Metabolite)		High selection risk of effluent at 1 WWTP in Australia; all measured influent concentrations exceeded $PNEC_R$.	[44,45]

2.2. Priority antimicrobials in agribusiness environments

Similarly to our approach for monitoring antimicrobials in aquatic systems, we propose a strategy-based approach for prioritising antimicrobials for monitoring in agribusiness environments based on two key stakeholder objectives:



To ensure relevance to Australia, **all compounds were searched in the APVMA PubCRIS database to confirm their registration or approval for use in Australia before inclusion in either strategy.** Compounds not registered for use in Australia were excluded from the agribusiness prioritisation framework.

For agribusiness stakeholders seeking a robust, condensed, internationally relevant and practical list of priority compounds for environmental monitoring, we have recommended following **internationally comprehensive guidelines** (Table 3).

This includes all antimicrobials listed as critically important antimicrobial agents on the WOAH's (World Organisation for Animal Health) List of Antimicrobial Agents of Veterinary Importance [47] and agriculturally relevant antimicrobials listed on the current EU Water Framework Directive Watch List (2025 [30]). Together these internationally recognised frameworks provide a practical and harmonised foundation for monitoring antimicrobials relevant to animal and plant production systems.

The WOAH List identifies antimicrobials of global importance to veterinary medicine and provides an internationally recognised reference for antimicrobial stewardship and surveillance. Aligning Strategy 1 with the WOAH List, together with agriculturally relevant antimicrobials included on the EU Water Framework Directive Watch List, supports international benchmarking while ensuring relevance across Australian animal and plant production systems.

For agribusiness stakeholders that prioritise national relevance, Strategy 2 (Table 4) expands the framework to include. analytes of particular interest for Australia. This list is informed by a horizon scan of nationally relevant evidence sources for antimicrobials of importance for Australian agribusiness systems and environments. To do this, key available resources were screened, including:

- All antimicrobials listed as 'High Importance' for animal health in: Australian Strategic and Technical Advisory Group on Antimicrobial Resistance (ASTAG) (2018). *Importance ratings and summary of antibacterial uses in human and animal health in Australia*. Canberra: Commonwealth of Australia.
- Australian Pesticides and Veterinary Medicines Authority (APVMA). (2014). Quantity of antimicrobial products sold for veterinary use in Australia (July 2005 to June 2010). Canberra, ACT: APVMA.
- Australian Wine Research Institute (2025). *Agrochemicals registered for use in Australian viticulture*. Adelaide: AWRI. Available at: https://www.awri.com.au/wp-content/uploads/agrochemical_booklet.pdf
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- Environment Protection Authority Tasmania (EPA Tasmania) (2026). *Florfenicol*. Available at: https://epa.tas.gov.au/business-industry/regulation/salmon-aquaculture/management-of-therapeutants-including-antibiotics/use-of-therapeutants-in-the-marine-environment/florfenicol

Table 4 should not be interpreted as a systematic, or exhaustive list. Rather, it provides a practical baseline that can be refined as additional Australia monitoring data and evidence become available.

***Table 3. International benchmarking priority compounds (International comprehensive) for environmental agribusiness monitoring in Australia**, adopted from WOAH's (World Organisation for Animal Health) List of Antimicrobial Agents of Veterinary Importance [47] and the 2025 EU Water Framework Directive's Watch List [30]. Only antimicrobials registered for use with the Australian Government's APVMA PubCRIS database [32] are included. Additional details provided by PubCRIS. **Emboldened text** highlights compounds included in our national relevance tier below.*

Compound	Type	Class	Comment	Source list
Spectinomycin	Antibacterial	Aminocyclitol	Used for respiratory and enteric infections in multiple species, including cats, dogs, pigs and poultry broilers.	WOAH
Dihydrostreptomycin		Aminoglycoside	Aminoglycosides are of importance in septicaemias; digestive, respiratory and urinary diseases. Dihydrostreptomycin is used to treat post-weaning bacterial enteritis in calves.	
Streptomycin				
Gentamicin			Gentamicin is an important medicine for *Pseudomonas aeruginosa* infections with few alternatives. Also used to treat bacterial infections in cats, dogs and horses. Specifically used to manage: *Actinobacillus spp., Bordetella bronchiseptica, Enterobacter spp., E. coli, Klebsiella spp., Pseudomonas spp.*	
Apramycin			Currently only used in animals. Apramycin is used to treat colibacillosis in broilers, and bacterial enteritis (*E. coli* or *Salmonella spp.*) in calves, horses and pigs.	
Florfenicol		Amphenicol	This class is of particular importance in treating some fish diseases, in which there are currently no or very few treatment alternatives. This class also represents a useful alternative in respiratory infections of cattle, swine and poultry.	

Ceftiofur	Cephalosporin	Cephalosporins are used in the treatment of septicaemias, respiratory infections, and mastitis. Alternatives are limited in efficacy through either inadequate spectrum or presence of antimicrobial resistance.	
Erythromycin	Macrolide	Macrolides are used to treat Mycoplasma infections in pigs and poultry, haemorrhagic digestive disease in pigs (*Lawsonia intracellularis*) and liver abscesses (*Fusobacterium necrophorum*) in cattle, where they have very few alternatives.	
Oleandomycin			
Tulathromycin			
Kitasamycin			
Spiramycin			
Tilmicosin			
Amoxycillin	Penicillins	This class is used in the treatment of septicaemias, respiratory and urinary tract infections. This class is very important in the treatment of many diseases in a broad range of animal species. Few economical alternatives are available.	
Ampicillin			
Amoxycillin + Clavulanic Acid			
Cloxacillin			
Phthalylsulfathiazole	Sulfonamide	These classes alone or in combination are critically important in the treatment of a wide range of diseases (bacterial, coccidia and protozoal infections) in a wide	
Sulfacetamide			

Sulfadiazine			range of animal species. Typically applied in combination therapy with trimethoprim.	
Sulfadimidine				
Sulfadoxine				
Sulfamerazine				
Sulfaquinoxaline				
Trimethoprim + Sulfonamide		Sulfonamides and diaminopyrimidine		
Chlortetracycline		Tetracycline	This class is critically important in the treatment of many bacterial and chlamydial diseases in a wide range of animal species. Also, critically important against *Anaplasma marginale* due to lack of antimicrobial alternatives.	
Doxycycline				
Oxytetracycline				WOAH and EU Watch List (2025)
Tetracycline				
Tylosin		Macrolide	As above, macrolides are used particularly in livestock (e.g. cattle, pigs and poultry). Sometimes used in companion animals. Treats respiratory and enteric infections. Tylosin is used to manage: bacterial infection, foot rot, leptospirosis, mastitis, metritis and pneumonia in cattle; dysentery, pneumonia and bacterial infection in pigs.	
Climbazole	Antifungal	Azole (imidazole)	Climbazole used as a fungicide in crop protection. Listed on the current European Watch List.	EU Watch

Cyazofamid		Azole (cyanoimidazole)	Cyazofamid used as a fungicide to manage *Phytophthora infestans* (late blight) in potatoes, white blister/white rust in broccoli and Pythium leaf blight in turf.	List (2025)
Difenoconazole		Azole (triazole)	Difenoconazole used as a fungicide to manage: black sigatoka, leaf spot or yellow sigatoka on bananas, leaf blight in carrots, husk spot (*Pseudocercospora macadamiae)* in macadamias, early blight in potatoes and target spot in tomatoes.	
Epoxiconazole			Epoxiconazole used as a fungicide to manage leaf rust (*Puccinia hordei*) in barley and leaf rust, blotch or stripe rust (*Septoria nordorum*) in wheat.	
Mefentrifluconazole			Mefentrifluconazole used as a fungicide to treat anthracnose (C*olletotrichum graminicola*), brown patch (*Rhizoctonia spp.*), dollar spot, spring dead spot, blossom blight (*Monilinia fructicola*) in almonds; Alternaria fruit spot in apples; gummy stem blight (*Didymella bryoniae*) and powdery mildew in cucurbits; powdery mildew in fruiting vegetables and grapes; and husk spot (*Pseudocercospora macadamiae*) in macadamias.	
Propiconazole			Propiconazole used fungicide to manage: apricots; bananas; barley; treat lawns; oats; peanuts; rust on peppermint; pineapples; poppies; prune rust; stone fruit; sugar cane and wheat.	
Triticonazole			Triticonazole used to manage turf: *Dreschslera spp., Fusarium spp., Helminthosporium spp., rhizoctonia spp; Tilletia spp.* in barley, oats and wheat.	

***Table 4. Australia-specific priority antimicrobial compounds (AU targeted) for environmental agribusiness monitoring in Australia**, identified by horizon scan. **Emboldened text** highlights compounds currently listed on WOAH's (List of Antimicrobial Agents of Veterinary Importance.*

Antimicrobial	Type	Class	Industry Priority	Reason	ref
Penconazole	Antifungal	Azole (triazole)	Horticulture; Viticulture	Restricted in Australian viticulture. Resistance detected in the national fungicide resistance testing program.	48
Azoxystrobin	Fungicide	Strobilurin		Resistance detected in the national fungicide resistance testing program in Australian viticulture.	
Pyraclostrobin		Strobilurin			
Proquinazid		Quinozolinone			
Quinoxyfen		Quinolines			
Metalaxyl		Acylalanine			
Pyraclostrobin		Strobilurin			
Pyrimethanil		Anilino-pyrimidine			
Fludioxonil		Phenylpyrrole			
Fenhexamid		Hydroxyanilide			
Florfenicol	Antibacterial	Amphenicol	Aquaculture and livestock	Detection of florfenicol metabolites in low levels in non-target/wild species following use in aquaculture (salmon farming).	13,49,50
Ceftriaxone		Cephalosporin	Companion animals	High importance antimicrobial (ASTAG) for use in exceptional circumstances.	51
Cefotaxime					

Ceftiofur			Livestock-related agriculture	High importance antimicrobial (ASTAG) approved for use with strict label restraints. Not used in humans but has potential to select for cross resistance to antibacterials used in humans.	
Cefovecin			Companion animals	High importance antimicrobial (ASTAG) approved with strict label restraints. Not used in humans but has potential to select for cross resistance to antibacterials used in humans.	
Sodium fusidate		Fusidane		High importance antimicrobial (ASTAG). Topical treatment use in skin or eye infections in dogs and cats.	
Nitrofurazone		Nitrofurans		High importance antimicrobial (ASTAG). Topical treatment for infections of skin and ear for horses, dogs and cats. All registrations in food-producing species cancelled in 1992. Can result in cross-resistance to human antibacterials.	
Polymyxin B		Polymyxin	Livestock-related agriculture and companion animals	High importance antimicrobial (ASTAG). Approved for topical ocular and aural use in cattle, sheep, horses, dogs and cats in exceptional circumstances.	
Enrofloxacin Enrofloxacin+ Silver Sulfadiazine		Quinolone	Companion animals	High importance antimicrobial (ASTAG). Approved for use in dogs and cats. Potential for cross resistance to human antibacterials. Label restraints including ‘do not use in food producing animals’ and ‘for use in companion animals where culture and sensitivity testing indicate no suitable alternative’. Only appropriate in exceptional circumstances.	
Ibaflocaxin					
Marbofloxacin					
Orbifloxacin					
Pradofloxacin					
Virginiamycin		Streptogramin	Livestock-related agriculture	High importance antimicrobial (ASTAG). Approved for use in cattle, sheep, broiler chickens and horses. Potential for cross resistance to human antibacterials. Label restraints e.g. 'do not feed to laying birds', ‘do not use in horses that may be slaughtered for human consumption’.	
Amoxycillin		Penicillins			52

Ampicillin			Livestock-related agriculture and companion animals	Approved for a variety of uses in both food producing and companion animals. Top 4 highest classes of antimicrobial sales (tonnes of active constituent) of veterinary antimicrobials used for therapeutic purposes in food animals (2005-2010, Australia). Highest class of antimicrobial sales (tonnes of active constituent) of veterinary antimicrobials used for non-food animals (2005-2010, Australia).	
Benzathine penicillin					
Amoxicillin + Clavulanic Acid					
Cloxacillin					
Penethamate					
Procaine penicillin					
Bacitracin		Polypeptides	Livestock-related agriculture and companion animals	Approved for a variety of uses in both food producing and companion animals, but mostly used for cats and dogs. Bacitracin widely used to treat necrotic enteritis due to *Clostridium perfringens*. Top 4 highest classes of antimicrobial sales (tonnes of active constituent) of veterinary antimicrobials used for therapeutic purposes in food animals (2005-2010, Australia).	
Polymyxin B					
Erythromycin		Macrolides	Livestock-related agriculture	Approved for use in a variety of food-producing animals. Top 4 highest classes of antimicrobial sales (tonnes of active constituent) of veterinary antimicrobials used for therapeutic purposes in food animals (2005-2010, Australia).	
Kitasamycin					
Oleandomycin					
Tilmicosin					
Tulathromycin					
Tylosin					
Chlortetracycline		Tetracyclines	Livestock-related agriculture and companion animals	Chlortetracycline and oxytetracycline used for both food-producing and companion animals. Doxycycline and tetracycline used for companion animals. Top 4 highest classes of antimicrobial sales (tonnes of active constituent) of veterinary antimicrobials used for therapeutic purposes in food animals (2005-2010, Australia).	
Doxycycline					
Oxytetracycline					
Tetracycline					

Potential AMR co-selective compounds

Whilst this report's scope and focus is limited to antimicrobials and their transformation products, other environmental compounds have also been shown to contribute to AMR co-selection under some conditions. Although these compounds fall outside the scope of this report, they represent an important area for future research and monitoring.

This list is not exhaustive and reflects the current evidence base, which continues to evolve. At present, there are no internationally coordinated monitoring frameworks specifically targeting AMR co-selective agents or adopting AMR selection endpoints.

Examples reported in the literature include:

- Copper [53]
- Zinc [54]
- Lead [55]
- Cadmium [56]
- Quaternary ammonium compounds [57]
- Biocides, including triclosan [58]
- Some non-antibacterial pharmaceuticals including diclofenac, metformin and 17-β-estradiol [29,59], have also been identified as potential co-selective agents, particularly in combination antimicrobial compounds[2,61].

2.1. A One Health prioritisation framework

In our initial prioritisation (Sections 2.1 and 2.2), we sought to account for the diverse factors shaping antimicrobial monitoring capacities across stakeholders, recognising that utilities, laboratories, and industries differ substantially in analytical capacity, infrastructure, and available resources.

However, sector-specific prioritisation alone does not fully capture the interconnected nature of antimicrobial occurrence and movement within a One Health framework. **Antimicrobials may move between aquatic and agribusiness environments through a range of interconnected pathways.** For example, recycled water may be used for irrigation, bio-solids may be beneficially reused on agricultural land, and sewer overflows may discharge to receiving environments, including those supporting aquaculture. Conversely, agribusiness activities may also contribute residual antimicrobials to aquatic environments through processes such as runoff to surface waters, for example.

Accordingly, we propose an integrated One Health monitoring framework (Figure 2). Where resources and monitoring objectives permit, coordinated monitoring across stakeholders would target antimicrobials identified as priorities across domestic wastewater, surface waters, horticulture, viticulture, aquaculture and livestock systems. This integrated approach provides the most comprehensive understanding of antimicrobial occurrence across interconnected One Health settings while supporting collaboration, data comparability and evidence-based decision-making. User guidance and supporting case studies are provided (Figure 3).

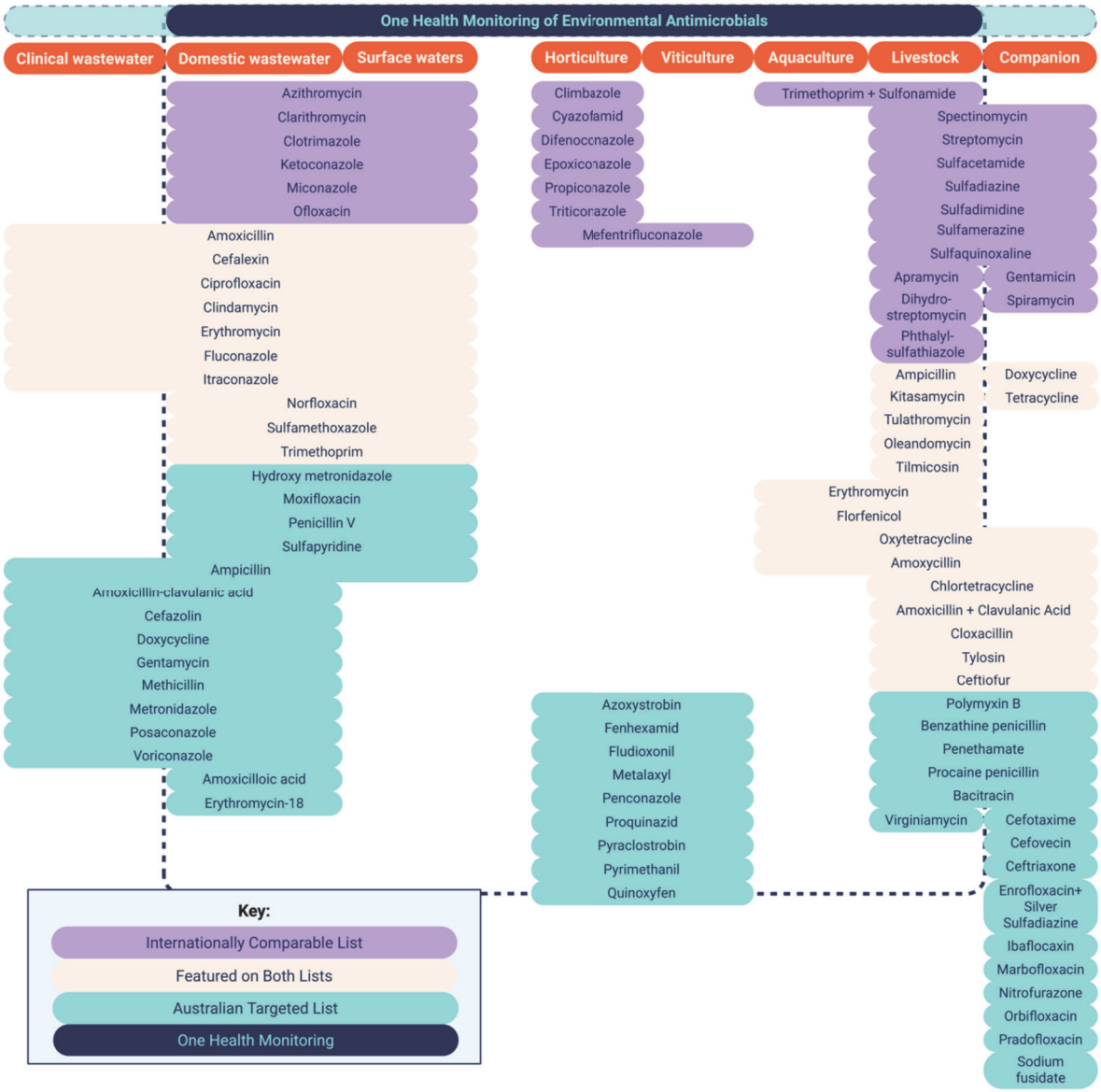

Figure 2. *A One Health prioritisation framework for environmental antimicrobial monitoring. Created using BioRender.com.*

The following examples (Figure 3) illustrate how the strategy-based prioritisation framework can be applied to different monitoring objectives. They are intended as examples only, and alternative approaches may be appropriate depending on monitoring goals, available resources and sector-specific requirements.

Since the completion of this report, the Quadripartite Joint Secretariat on AMR generated a comprehensive list of antimicrobial classes and subclasses proposed for an antimicrobial panel for testing bacteria in an integrated One Health surveillance programme. Several of the antimicrobials listed in Figure 2 are included. For more details, please see the full report:

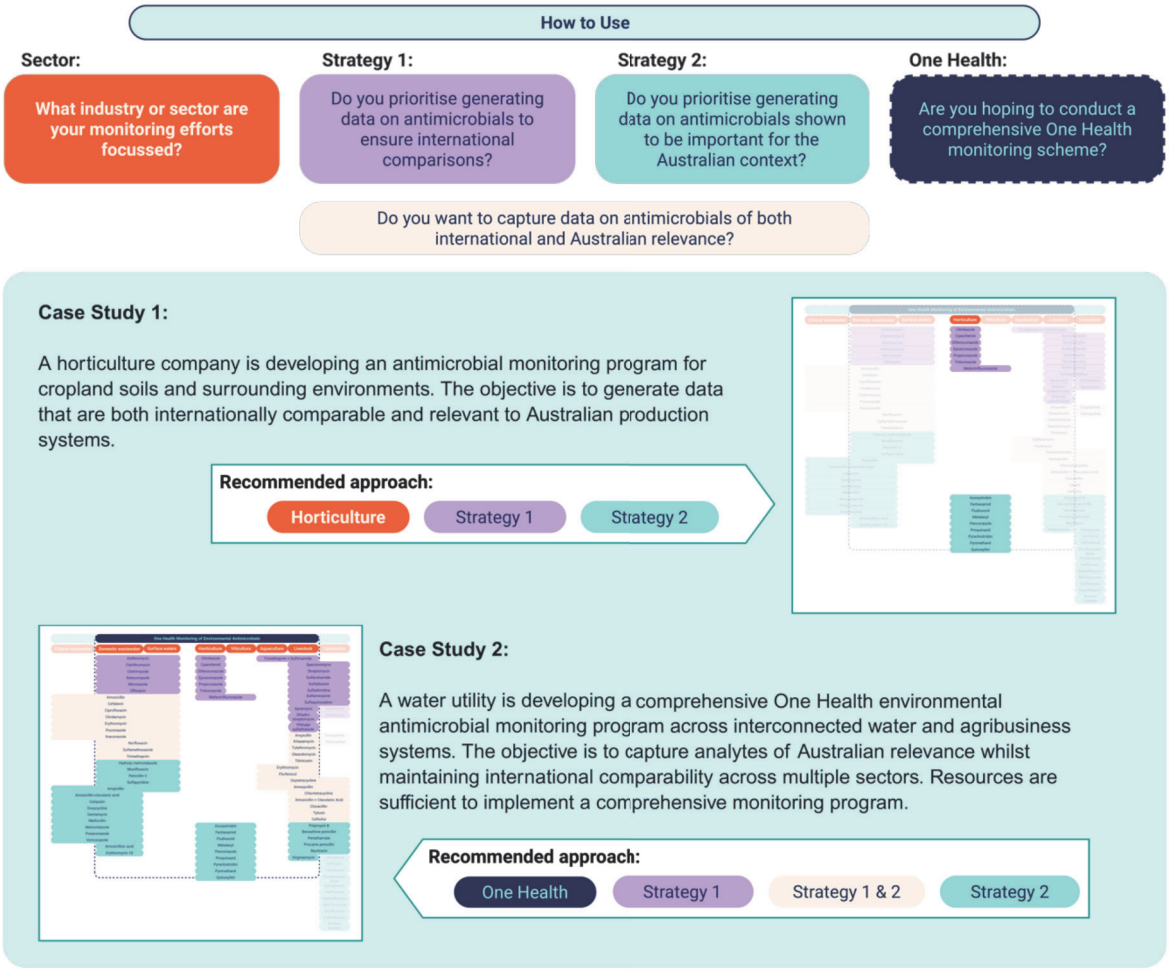

Figure 3. *Using the One Health prioritisation framework for environmental antimicrobial monitoring, with supporting case studies. Created using BioRender.com.*

Quadripartite guidance on One Health integrated surveillance of antimicrobial resistance and use. Geneva: World Health Organization, Food and Agriculture Organization of the United Nations, United Nations Environment Programme and World Organisation for Animal Health; 2025. Licence: CC BY-NC-SA 3.0 IGO.

3. Core Antimicrobial Monitoring Methods

When deciding upon an appropriate detection method, sensitivity (i.e. limits of detection and quantification), specificity and suitability are all essential to consider. Likewise, the preferred method will depend on the sample matrix, access to analytical instrumentation, and the monitoring objectives. Ultimately, method selection should be guided by the research question, monitoring objective and application of the data.

Recommendations of when to use certain methods are shown in the decision-making framework below (Figure 4), with illustrative case studies provided (Figure 5). The strengths and limitations of each method, together with their recommended applications, are discussed throughout this section (Table 5).

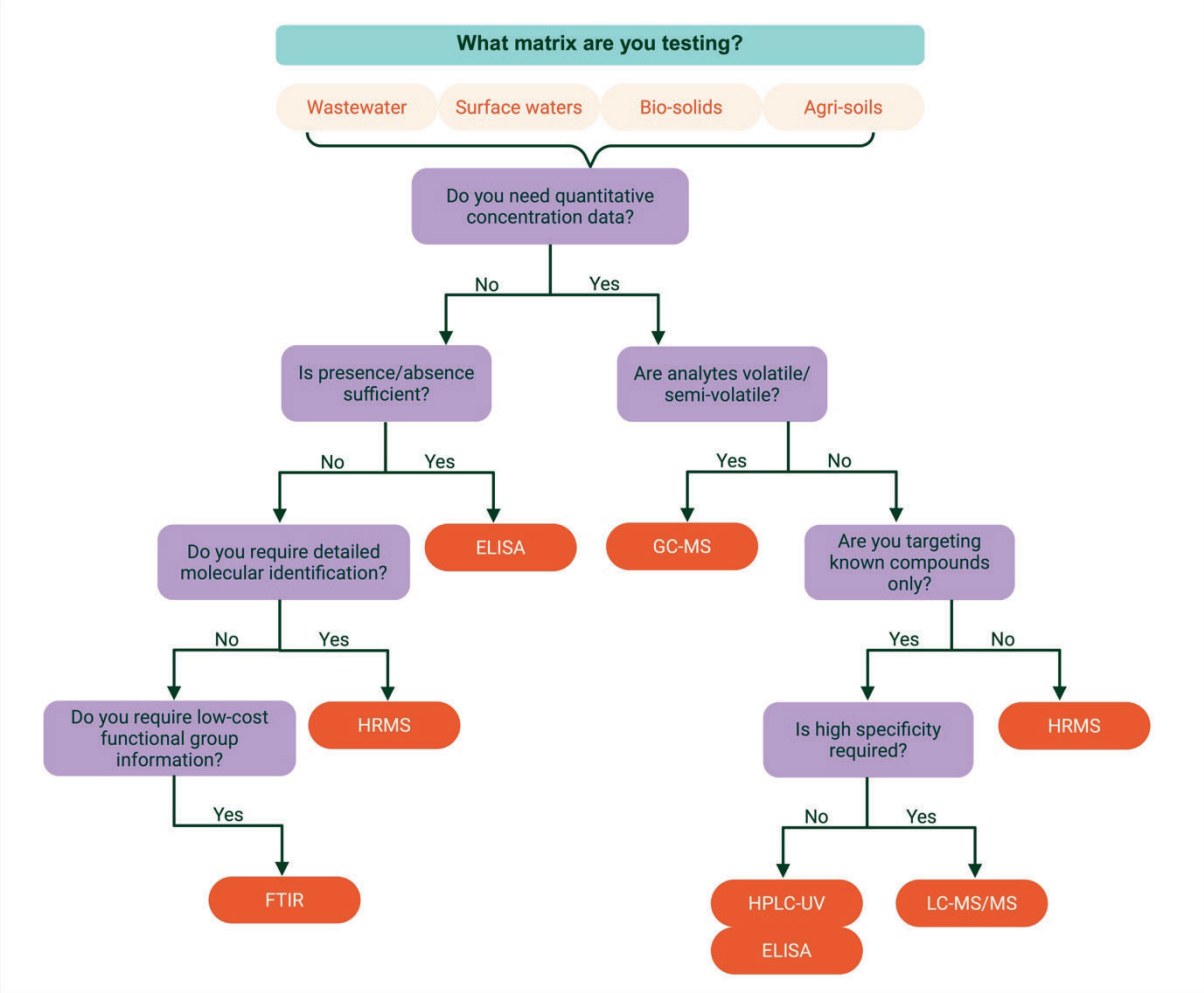

Figure 4. *Simplified decision-making framework for environmental antimicrobial monitoring. Created using BioRender.com.*

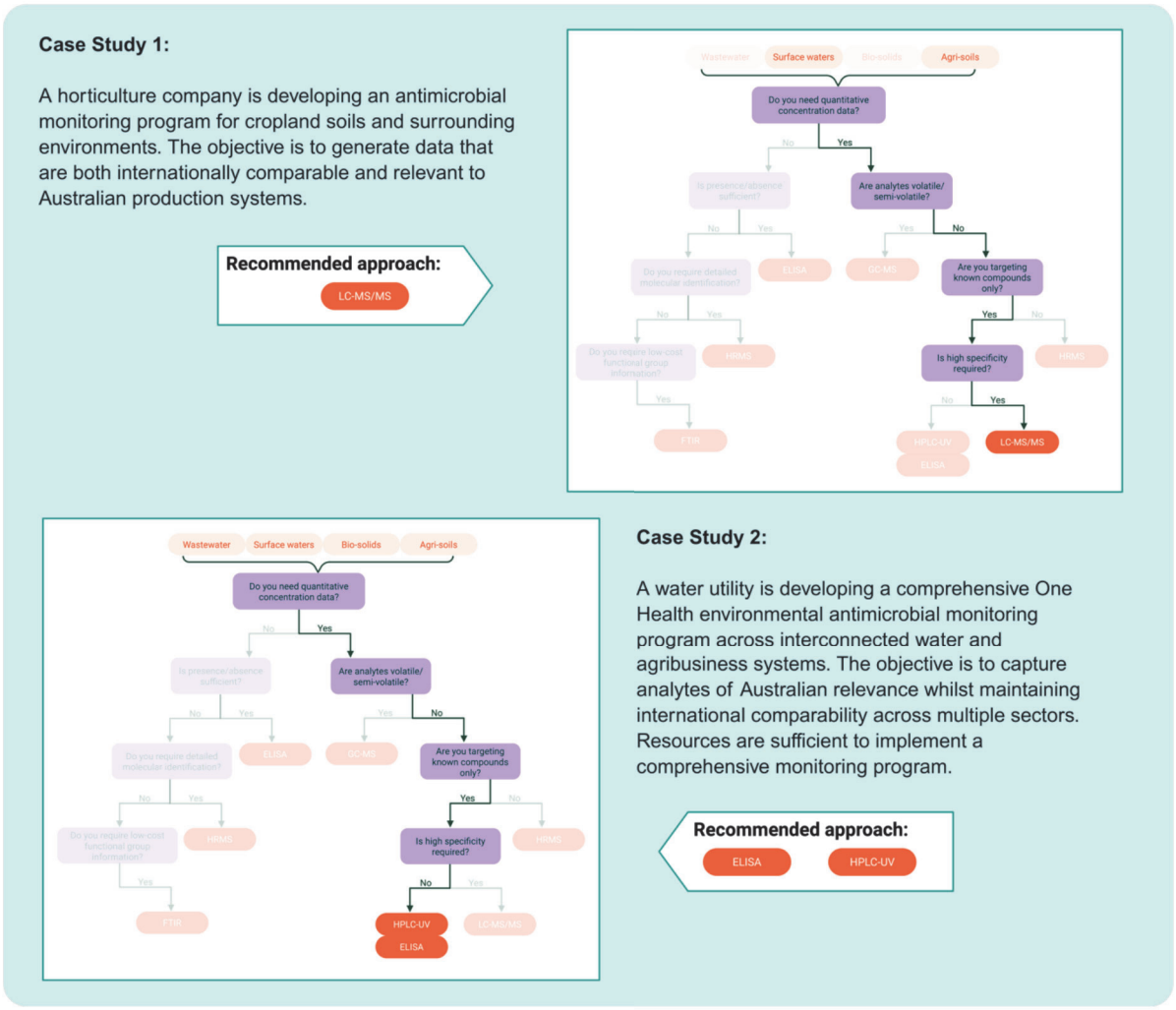

Figure 5. *Using the decision-making framework for environmental antimicrobial monitoring, with supporting case studies. Created using BioRender.com.*

3.1. Overview of conventional analytical techniques and detection methods

Table 5. *Summary of antimicrobial detection methods, with strengths and weaknesses of use.*

Method	Principle	Simplified methodology	Strengths	Weaknesses	REFs
Liquid Chromatography-Tandem Mass Spectrometry (**LC-MS/MS OR LC-MS^2**)	Antibacterials (analyte) within a sample are chemically separated via interaction with a stationary phase, ionised, fragmented and then analysed based on the mass-to-charge ratio of the ions.	Liquid samples are injected into a mobile phase which passes through a column containing a 'stationary phase'. Analytes are separated based on polarity, hydrophobicity, charge, and size, and are ionised before entering the mass spectrometer for quantification. Commonly used to detect antimicrobials in wastewater, surface water, drinking water or to inform environmental risk assessments.	Highly sensitive and selective; excellent quantification; able to confirm specific compounds; allows separation and detection of compounds with the same molecular mass but different product ions, even if they co-elute; less complicated sample preparation than GC-MS; can be used to detect multiple analytes in complex samples; broad analytical scope.	High cost of instrumentation and maintenance; requires relatively skilled operators; potential for matrix effects; some workflows require extensive sample preparation; suppression of electrospray ionisation likely to occur in highly contaminated samples (sewage), so efficient clean-up step needed or appropriate standard required.	[3,60–62]

High-performance Liquid Chromatography with Ultraviolet Detection (**HPLC-UV**)	Analytes are separated following conventional chromatography, followed by a UV detector which uses UV wavelengths according to Beer-Lamber law to deduce proportional concentrations.	Liquid samples pass through liquid chromatography step as above, producing a chromatogram. However, as samples exit the column, they pass through a UV detector, emitting light through a flow cell. Analytes absorb UV wavelengths and the instrument coverts changes in light to a measurable signal proportional to concentration. Commonly used to screen for antimicrobials in environmental samples when high precision isn't required.	Simple; low cost; routinely used; precise quantification; suitable for a wide range of analytes; require less maintenance than mass spectrometers.	Not suitable for non-UV-active compounds; not as sensitive as mass spectrometry; may be influenced by matrix interference; does not provide structural information.	63
Gas Chromatography-Mass Spectrometry (**GC-MS**)	Detection and quantification of analytes follow the same principle as LC-MS, but samples are first vaporised and carried by an inert gas through a 'capillary column'.	Samples are first vaporised through a capillary column and coated with the stationary phase, then are separate based on volatility and polarity, eluting at unique retention times. From this, separated analytes enter the mass spectrometer which ionises the molecules and fragments them. Commonly used to detect and monitor antimicrobials in soils or sediments.	Good for volatile/derivatised drugs; provides structural information of the compound; widely adopted and optimised around the world.	Prior derivatisation of polar pharmaceuticals is necessary using highly toxic or carcinogenic reagents; this step may also affect the accuracy of this method; high instrument and maintenance costs; fairly limited to low molecular weight compounds.	60,64

High-Resolution Mass Spectrometry (**HRMS**)	Distinction lies in resolution of analyser, regardless of type of chromatography used prior to analyses. This method uses instruments with high resolving power, meaning exact mass is measured.	Analyte molecules are converted into gas-phase ions using electrospray ionisation or MALDI. These ions are then separated according to their mass-to-charge ratio, with extremely high resolution. This enables the measurement to exact mass, producing highly accurate mass spectra. Commonly used to discover previously unmonitored antimicrobials and transformation products in environmental samples.	Finds 'unknowns'; high mass accuracy; can discriminate ions that differ by a fraction of a Dalton; higher confidence in molecular formula assignment; provides 'molecular fingerprints'; high resolving power enables separation of nearly isobaric ions.	Expensive; outputs require advanced computational tools and expertise for interpretation; some samples require tedious sample preparation.	[65]
Enzyme-linked Immunosorbent Assays (**ELISA**)	ELISA is an immunoassay which relies on antigen-antibody interactions to detect and quantify biomolecules. For antibacterial detection, ELISA adopts antibacterial-protein conjugates.	Capture antigens or antibodies are immobilised onto a solid surface (usually a microplate). When your sample is added, target molecules (if present) will bind to the capture molecule. A detection antibody is then added, followed by a colour-producing reagent, creating a measurable signal (colour change) which reflects the analytes concentration. Commonly used for on-site/in-field screening for antimicrobials.	Rapid, high-throughput screening; relatively sensitive; can be quantitative (absorbance) or qualitative (presence/absence); highly scalable; extremely versatile.	Risk of non-specific binding; cannot reveal any information about molecular structure like mass spectrometry.	[66]

Fourier Transform Infrared Spectroscopy (**FTIR**)	Antibacterials (and other compounds) have distinct infrared absorption patterns in the fingerprint region, allowing quantification monitoring through spectral intensity changes.	FTIR measures how antibacterial molecules absorb infrared light at characteristic vibrational frequency. Using infrared fingerprints from known standards (produced by vibrations of chemical bonds), antimicrobials can be identified and quantified (using peak intensity or area under the curve). Commonly used to identify antimicrobials in polluted environments.	Quick structural fingerprints; reagent free; minimal sample preparation required. Attenuated Total Reflection (ATR-FTIR) is particularly useful as it allows direct measurement with litter or no sample prep; also possess capacity for bacterial typing, with benefits for AMR surveillance.	Some antibacterials have very similar infrared spectra, so differentiation can be difficult; requires advanced data analysis; less sensitive than mass spectroscopy; standards required (cannot be used for unknowns).	67,68

3.2. Chromatographic methods

3.2.1. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) remains the gold standard for reliable identification and quantification of non-volatile compounds. This method relies on the separation of analytes using liquid chromatography, which are then ionised, fragmented and analysed based on the mass-to-charge ratio of the ions (Table 5). Liquid Chromatography-Tandem Mass Spectrometry methodologies are known to be extremely sensitive and selective, providing excellent quantification using Triple Quadrupole (QQQ); Quadrupole-Time-of-Flight (Q-TOF); Ion Trap or Orbitrap mass analysers. This method is known for its ability to detect very low concentrations of antimicrobials, which is essential, given that even extremely low, sub-inhibitory concentrations can select for AMR [69].

A key benefit of LC-MS/MS is its multi-analyte capacity, with one previous method developed to analyse 31 different antimicrobials – including macrolides, quinolones, sulfonamides and tetracyclines – in WWTP effluents [70]. Another article recently described a high-throughput method to detect over 100 antimicrobials and metabolites [44,45,61]. This method has since been validated using Australian influent and effluent samples, and has several prospects for its capacity in improving environmental antimicrobial monitoring (Figure 6).

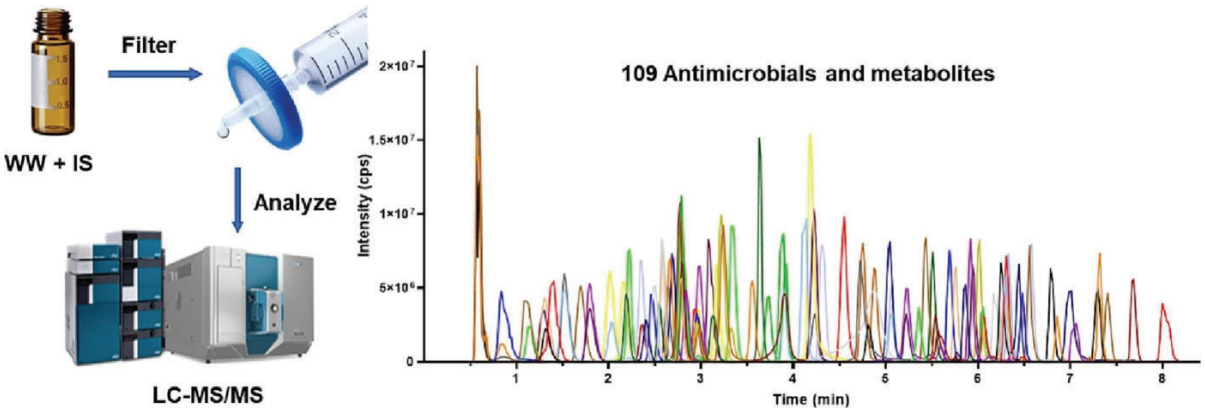

Figure 6. *Summary of method and extracted ion chromatograms of all compounds in Li et al. [61]. WW: wastewater; IS: internal standard.*

Whilst LC-MS/MS can be extremely beneficial, the high cost of instrumentation and maintenance reduces accessibility for laboratories across the globe, especially in regional settings. Beyond practicalities of LC-MS/MS, suppression of electrospray ionisation is likely to occur in highly contaminated samples, including sewage. This means that efficient clean-up is essential and may extend the workflow and expense of the process.

3.2.2. High-Performance Liquid Chromatography with Ultraviolet Detection (HPLC-UV)

High-performance liquid chromatography with ultraviolet detection (HPLC-UV) follows the same starting process as above, where analytes are separated using liquid chromatography instruments and technologies (Figure 7). However, this method differs in that analytes are distinguished based on absorption of UV wavelengths proportional to concentration (Table 5).

This method is arguably more simple and therefore lower cost than LC-MS/MS, granting precise quantification and requiring less maintenance than mass spectrometers. However, HPLC-UV is somewhat restricted, given that it cannot be used to quantify non-UV active compounds, nor does it provide structural information of the compound, and is more prone to interference.

Using HPLC-UV, a recent paper developed and validated a solid-phase extraction (SPE) coupled with HPLC-UV protocol to sensitively detect cefoperazone, cefipime, ceftazidime, ceftriaxone, cefdinir, cefotaxime, amoxicillin, levofloxacin and ciprofloxacin in water samples; a method which could be adopted to quantify antimicrobials in wastewater (albeit less sensitive than LC-MS/MS) [71].

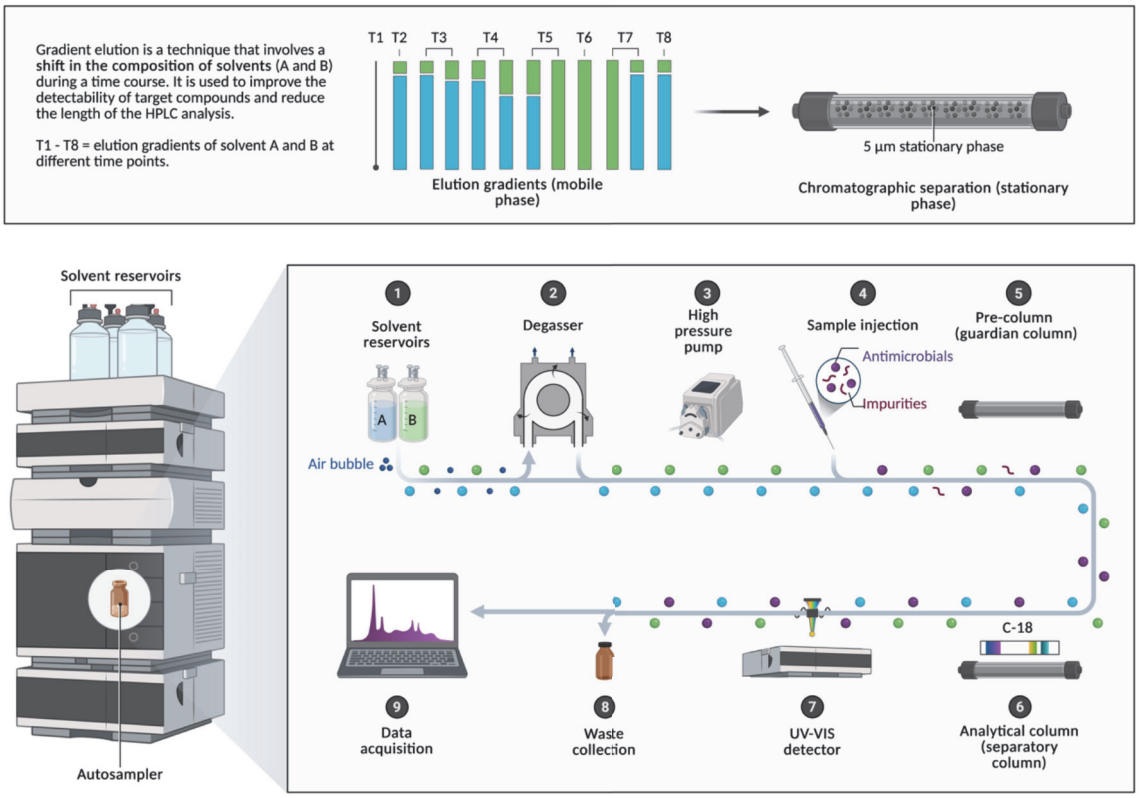

Figure 7. *Components and steps of HPLC-UV analysis. Created in https://BioRender.com by Grozic and Kim [72,73].*

3.2.3. Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) follows the same mass spectrometry principles as LC-MS, but samples are instead vaporised and carried by an inert gas and separated using gas chromatography (Figure 8).

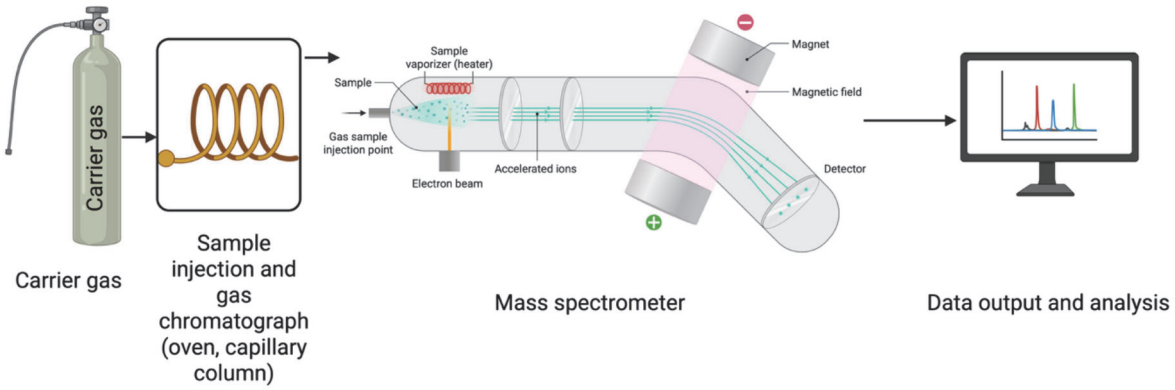

Figure 8. *Schematic diagram summarising GC-MS process. Created in https://BioRender.com.*

It should be noted here that the majority of pharmaceuticals lack sufficient volatility and are therefore not directly compatible with GC [74], however, this can often be rectified via derivatisation [75].

A novel method was published in 2015 which adopts GC-MS for the simultaneous determination of triclosan, a pervasive antimicrobial agent - and it's transformation products: 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and methyl triclosan - from both wastewater and sludge samples [76]. Using this method, researchers revealed that the concentration of each transformation product varied according to the type of treatment processes and sludge facilities present, revealing that analytical methods not only need to consider the matrix and analyte characteristics, but also the context of which the sample was extracted.

3.3. High-resolution and advanced mass spectrometry

3.3.1. High-Resolution Mass Spectrometry (HRMS)

Whilst this method is indeed mass spectrometry, the distinction lies in the resolution of the analyser, regardless of any prior chromatography. High-Resolution Mass Spectrometry (HRMS) is particularly beneficial when suspect screening, or conducting non-target screening for unknowns, given the high resolving power measuring exact mass of analytes. However, with this comes great financial burden, and requirement for advanced computational tools (Table 5).

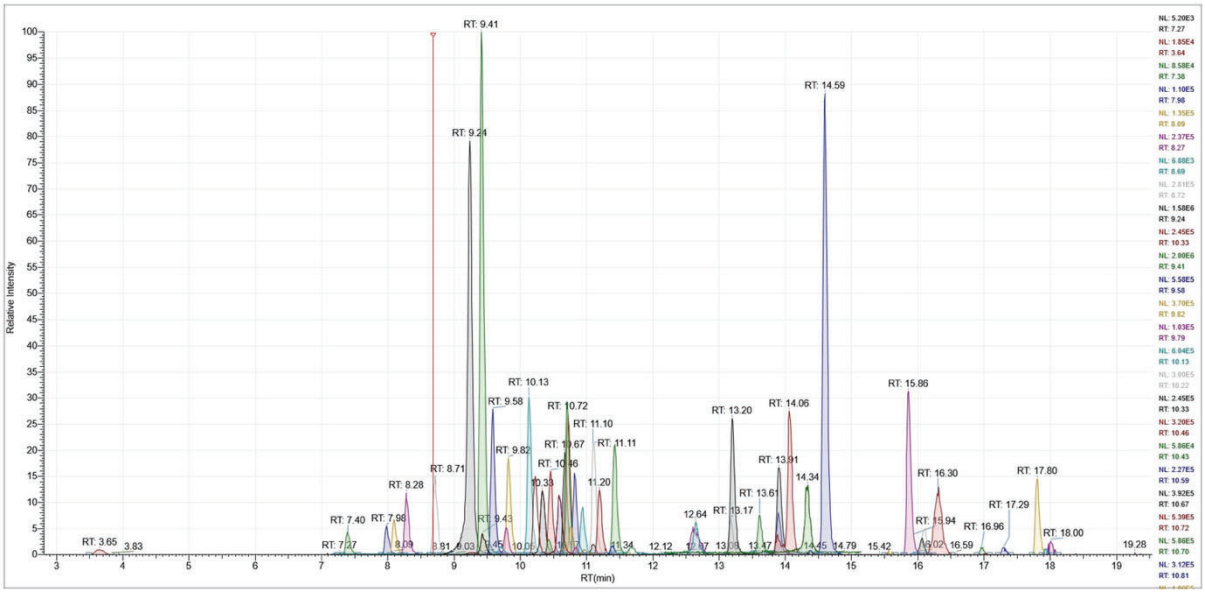

Figure 9. *HRMS chromatogram of a milk extract fortified at screening target concentration level for 57 antimicrobial compounds [77].*

Like LC-MS/MS and LC-UV, a benefit of HRMS is the capacity for multiresidue analyses. Furthermore, HRMS can provide additional accurate mass information of precursor ions and fragments which can help in the tentative identification (i.e. tentative structure) of unknowns, particularly when you don't have a reference standard available.

Previous work developed a multi-analyte method for the determination of antibacterials in milk, compliant with procedures established by the European Union Commission Implementing Regulation 2021/808 (outlining maximum residue limits (MRLs) for food contaminants) [77]. In total, 57 different antibacterials (including beta-lactams, tetracyclines, sulfonamides, quinolones, pleuromutilins, macrolides and lincosamides) were successfully quantified in milk samples (Figure 9). This method exemplifies potential applications for HRMS in monitoring antimicrobials in environmental, liquid samples.

Building on this, a recent study (2024) produced a further HRMS-based analytical protocol to aid the monitoring antimicrobials. Specifically, this method is able to simultaneously detect 214 veterinary drugs at limits of detection of 0.1-10 ng/g [78]. Again, this protocol has great potential in aiding the monitoring of antimicrobials in diverse matrices.

3.4. Immunoassays and biosensors

3.4.1. Enzyme-linked Immunosorbent Assays (ELISA)

The 'ELISA' (enzyme-linked immunosorbent assay) is an immunoassay which relies on antigen-antibody interactions to detect and quantify biomolecules. For antibacterial detection and quantification, ELISA adopts antibacterial-protein conjugates. This method is extremely beneficial in its speed, high-throughput methodology and scalability. Its downfalls are that the ELISA cannot provide information on chemical structure but is a great option for simple monitoring and quantification work, especially for lower income settings.

Furthermore, in comparison to the abovementioned tools, ELISA requires significantly less sample cleanup, reducing the time and demands of the workflow. The principle of the ELISA is based upon the production of a colour-producing reagent, where the colour change is a measurable signal proportional to analyte concentration.

A previous study from Mosul City, Iraq, conducted an environmental health risk assessment of antibacterials in sewage using an ELISA protocol [79]. Within this work, a standard ELISA 'kit' was purchased from a private company and utilised following manufacturers guidelines, detecting antibacterials in all samples. This clearly demonstrates the accessibility, versatility and simplicity in this method, alongside the practical capabilities of its data outputs.

Another study assessed antimicrobials in feed and fish from an aquaculture facility using ELISA kits [66] in Sicily, Italy. Specifically, 30 samples were tested for concentrations of chloramphenicol, gentamicin, enrofloxacin and the metabolites of furaltadone and furazolidone antibacterials. All analytes were detected in every sample, with gentamicin and chloramphenicol having the highest mean concentrations. Again, this study adopted commercial ELISA kits, demonstrating the applicability and accessibility of this method for a wide range of samples.

3.5. Spectroscopic and electrochemical methods

3.5.1. Fourier Transform Infrared (FTIR) or Raman Spectroscopy

Lastly, FTIR or Fourier Transform Infrared Spectroscopy is another beneficial methodology to have in an antimicrobial monitoring toolkit. This method relies on infrared spectra of antibacterial chemicals to produce reagent free, quick, structural fingerprints (Table 5). This method has been especially productive in previous antibacterial degradation assays [67], and can be used to measure antimicrobial quantities by analysing changes in absorbance intensity.

3.6. Potential methodological challenges

Some antimicrobials are more likely to be detected in certain matrices and not others, simply because of different chemical properties and partitioning. Additionally, there are matrix specific challenges to consider:

In liquid (surface waters, freshwater, marine and wastewater)

As with any sample type in analytical chemistry, matrix effects can interfere with analysis. This is, however, especially an issue for wastewater or highly polluted water samples [80]. With contamination comes high organic loads, suspended soils, microbial activity, other chemical contaminants and the presence of transformation products or metabolites. In attempt to overcome this, liquid samples typically require a pre-filtration or centrifugation step, which can increase workload and processing time, but will reduce contamination of samples. This is further compounded by the often very low concentrations of target analytes. If any analyte is lost in ineffective filtration, centrifugation, transport or storage, it could result in false negative results. To overcome this, isotopically labelled internal standards can be used [60]. Finally, a lack of standardised protocols between laboratories or countries limits the ability for inter- or intra-country / nation comparisons.

In solids (soils, agricultural waste and receiving sediments)

Analytical detection of antimicrobials in solid matrices – e.g. soils or solid animal waste - is also challenging due to the large number of compounds (both target or non-target) potentially present, and the need to distinguish between parent and metabolites. Antimicrobials in solid samples also often occur at very low concentrations and within complex, highly variable matrices. All of which further complicate extraction and cleanup, in samples that are rarely homogenous, requiring additional pre-treatment steps. Finally, cross-state comparisons and collaborations could be challenged by biosecurity permits, especially considering the transportation of soils or microbiologically active bio-solids.

4. Considerations for Future Development

Australia has strong foundations in antimicrobial stewardship, One Health policy and environmental monitoring. Building on these strengths presents opportunities to further enhance monitoring capability, improve data comparability and support evidence-based decision-making across agribusiness, water and environmental systems.

4.1. Where Australia can move next

Three short opportunities would support Australia's leadership:

Coordinated monitoring

Current monitoring primarily focuses on parent antimicrobial compounds. Expanding monitoring to include metabolites and transformation products, together with greater harmonisation of methods across sectors, would improve understanding of antimicrobial occurrence and enable more comparable datasets over time.

Broader evidence generation

As monitoring datasets mature, there will be opportunities to broaden the evidence base beyond parent compounds and conventional assessment endpoints. This may include improved understanding of environmental pathways, transformation products, co-selective compounds and One Health interactions across environmental, agricultural and water systems.

Practical Implementation

Continued advances in analytical methods and field-deployable technologies provide opportunities to improve accessibility, reduce analytical costs and support more routine monitoring across sectors.

Considerations for Future Development

Future work may include:

- refinement of priority analyte lists as new evidence emerges
- improved harmonisation of analytical methods
- inclusion of metabolites and transformation products
- development of nationally comparable datasets
- assessment of co-selective compounds
- continued collaboration across One Health sectors

5. Conclusions

Australia's national strategy against AMR appropriately recognises the importance of a One Health approach, providing a strong foundation for coordinated environmental antimicrobial monitoring. This report builds on that foundation by presenting a practical framework to support the prioritisation of antimicrobials, selection of analytical methods and implementation of monitoring across aquatic and agribusiness environments.

The uses of antimicrobials are complex, with different sectors adopting different classes and patterns of use. Therefore, prioritising analytes for monitoring requires consideration of sector-specific objectives, available resources and intended monitoring outcomes. In this report, we have presented prioritised antimicrobial lists together with the rationale for their inclusion. To account for variations in budget, scope and capacity, each list is organised by sector (aquatic or agribusiness) and monitoring strategy (international benchmarking or Australian targeted).

We also provide a One Health monitoring framework, to support coordinated monitoring across interconnected environmental settings. Together, these resources provide a practical foundation that can be refined as new evidence and monitoring data become available.

We also outline the analytical methods available to support antimicrobial monitoring, alongside a simplified decision-making framework to guide method selection across different monitoring scenarios.

Collectively, the frameworks, priority lists and guidance presented in this report provide a practical and adaptable foundation for environmental antimicrobial monitoring in Australia, supporting evidence generation, international benchmarking and informed One Health decision-making.

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SAAFE CRC

www.crcsaafe.com.au
enquiries@crcsaafe.com.au
+61 8 8302 3617
PO BOX 66, Pooraka 5095

Australian Government
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