Environmental Contamination: Causes and Solutions
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Review

Environmental Antimicrobial Resistance: Current Status and Future Prospects

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How To Cite: Zhai, N.; Thomas, K.V.; Li, J.; et al. Environmental Antimicrobial Resistance: Current Status and Future Prospects. *Environmental Contamination: Causes and Solutions* **2025**, *1*(1), 6. https://doi.org/10.53941/eccs.2025.100006

Received: 19 June 2025
Revised: 28 September 2025
Accepted: 10 October 2025
Published: 4 November 2025

Abstract: Antibiotics underpin modern medicine and food production, but their indiscriminate use has accelerated antimicrobial resistance (AMR). Here we profile ARG dissemination in water, soil, air and anthropogenic niches such as wastewater treatment plants, intensive farms and landfills. We show that sulfonamide resistance genes (*sul1/sul2*) dominate aquatic systems, while tetracycline and macrolide genes prevail in livestock environments. Emerging evidence links airborne ARGs to seasonal PM2.5 peaks and long range dust transport. We evaluate state of the art detection platforms—high throughput qPCR, Hi-C metagenomics, nanopore long reads and CRISPR-Cas diagnostics—and discuss their complementarity. Finally, we outline integrated One Health policies that couple real time genomic surveillance with antibiotic stewardship incentives, and spotlight novel agents such as gepotidacin and sulopenem that help address the innovation gap. Coordinated adoption of these strategies is essential to avert a post-antibiotic era. Global cooperation and forward-looking One Health frameworks will be crucial to meeting this challenge.

Keywords: antibiotic; antibiotic resistance gene; AMR in environment; metagenomics

Highlights:

- Integrates Global Antimicrobial Resistance and Use Surveillance System (GLASS) and Organisation for Economic Co-operation and Development (OECD) data to map global antibiotic consumption and resistance trends.
- Quantifies public-health and economic burdens of antimicrobial resistance and identifies stewardship gaps in low- and middle-income countries.
- Compares antibiotic-resistance gene (ARG) prevalence across water, soil, air and built environments.
- Appraises next-generation antibiotic-resistance gene (ARG) surveillance tools (long-read metagenomics, CRISPR diagnostics, artificial intelligence (AI)-enabled forecasting).
- Recommends One-Health interventions spanning regulatory, technological and behavioural domains.

1. Introduction

Antibiotics have underpinned modern medicine and food security for more than eight decades, turning lethal infections into routine clinical encounters and adding an estimated 20 years to global life expectancy [1,2]. Yet success has sown the seeds of obsolescence: bacteria, equipped with short generation times and expansive mobile gene pools, now thwart every major drug class. The Institute for Health Metrics and Evaluation (IHME) estimated that AMR caused 1.27 million deaths in 2019 and contributed to another 4.95 million—a toll that already exceeds HIV/AIDS and malaria [3]. Updated Global Antimicrobial Resistance and Use Surveillance System (GLASS) data released in September 2024 show third-generation cephalosporin resistance in *Klebsiella pneumoniae* exceeding

50% in 37 countries, while fluoroquinolone resistance in *Escherichia coli* tops 65% across South Asia [4]. Without decisive action, macro-economic models project 10 million AMR deaths per year and a cumulative 100 trillion USD loss to global GDP by 2050 [5]. Several factors explain why resistance is accelerating. First, antibiotic consumption continues to rise sharply. Human use increased from 21.1 to 34.8 billion defined daily doses (DDD) between 2000–2018; India, China, and the United States account for 51% of the volume [6]. Over-the-counter (OTC) sales still make up 42% of human antibiotics in India and 58% in parts of sub-Saharan Africa [7]. Livestock and aquaculture consumed 116,000 t of active ingredients in 2022 and are projected to rise by 8% by 2030, largely for growth promotion [8,9]. Second, innovation and stewardship have not kept pace with the escalating threat. Since 2016, the FDA has granted 27 QIDP/LPAD designations, yet only two first-in-class agents—gepotidacin (2024) and sulopenem/probenecid (2024)—have reached market [10]. Meanwhile, fewer than half of GLASS nations enforce hospital stewardship audits [4]. Third, environmental amplification accelerates resistance spread. Horizontal-gene-transfer hotspots in wastewater treatment plants (WWTPs), landfills, manure-amended soils, and urban aerosols bridge clinical and environmental reservoirs. The *mcr-1* colistin-resistance gene, first detected in Chinese swine in 2015, is now found on every continent—including Antarctic bird isolates—demonstrating rapid cross-ecosystem travel [11]. Finally, major blind spots remain in global surveillance. According to the WHO GLASS Report 2023, only 36 of 127 enrolled states had uploaded continuous laboratory data covering at least two years, and data completeness remained lowest in low-income settings [4]. Environmental surveillance is still rarer: 17 countries have ARGs or antibiotic residues in national water-quality programmes, and metadata standards diverge widely [12].

2. Mechanisms and Drivers of AMR

AMR is the end-product of intertwined genetic, biochemical, and ecological processes that enable microorganisms to withstand otherwise lethal drug exposures. Understanding these mechanisms is essential for designing interventions that remain effective across clinical, veterinary, and environmental settings. Figure 1 illustrates this cyclical dynamic: anthropogenic pressures such as antibiotic and metal contamination create selective gradients that favour adaptive microbial traits and mutation bursts. These, in turn, facilitate the spread of mobile genetic elements (MGEs) carrying stable ARGs, which ultimately feed back into clinical settings, driving treatment failure and further drug use [13–15]. Breaking this loop requires coordinated interventions targeting microbial genetics, environmental reservoirs, and human behaviour.

2.1. Genetic Basis of Resistance

At the genetic level, several adaptive mechanisms provide initial footholds for resistance. For example, sub-inhibitory fluoroquinolone exposure can up-regulate efflux pumps via the *marA/soxS* regulons, while membrane remodelling (such as lipopolysaccharide palmitoylation in *Pseudomonas*) transiently raises minimum inhibitory concentrations (MICs) of colistin [16]. Persister formation and biofilm development further protect bacterial populations by slowing metabolism and embedding cells within extracellular polymeric substances. Although these phenotypes often revert once drug pressure subsides, they provide a foundation for the subsequent acquisition of stable ARGs [17–19]. Vertical gene transfer (VGT) transmits chromosomal mutations from parent to progeny and underlies the stepwise accumulation of target-site substitutions, such as *gyrA/parC* alterations that confer fluoroquinolone resistance in *Escherichia coli* [20]. Horizontal gene transfer (HGT) accelerates dissemination across species boundaries through three principal routes: conjugation, transformation, and transduction.

Conjugation involves self-transmissible or mobilizable plasmids, integrative conjugative elements (ICEs), and genomic islands that mobilize cassettes such as *blaCTX-M*, *mcr-1*, and *tet(X)* [21,22].

Transformation occurs when naturally competent genera such as *Acinetobacter* and *Streptococcus scavenge* naked DNA and incorporate it via homologous recombination, a process facilitated within biofilms where extracellular DNA is abundant [23].

Transduction allows bacteriophages to package host DNA and deliver ARGs such as *dfrA* and *ermB* into new hosts [23].

MGEs including transposons, insertion sequences, and class 1 integrons integrate these processes, providing promoters and recombination sites that enhance ARG capture and expression [24]. Recent long-read metagenomic analyses have uncovered complex multireplicon plasmids carrying up to 15 ARG types flanked by *IS26* clusters—structures now reported in hospitals, livestock, and river water [25,26].

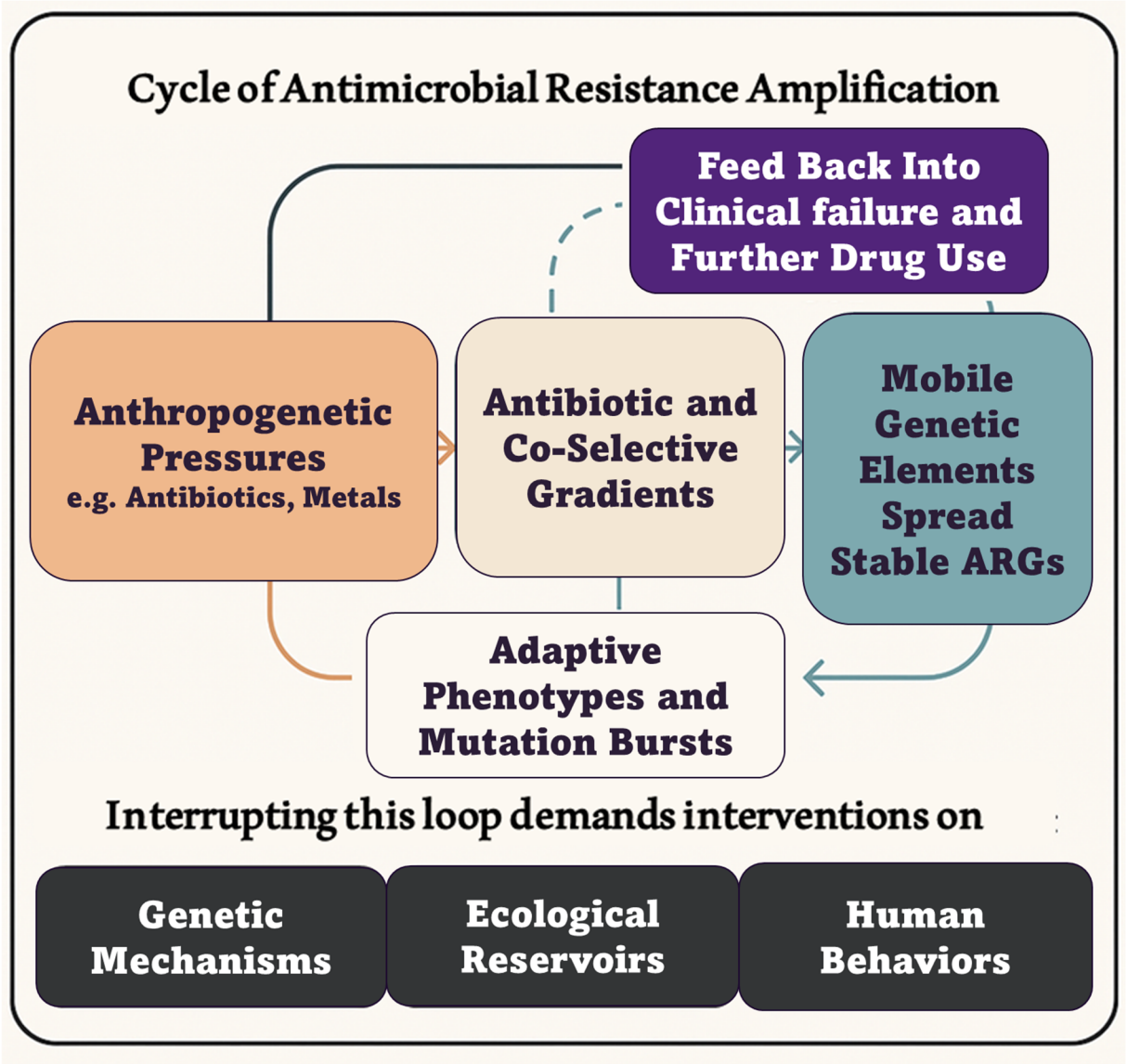

Figure 1. Cycle of antimicrobial resistance (AMR) amplification. Anthropogenic pressures such as antibiotics and metals (orange box) create selective gradients (beige box) that enrich adaptive phenotypes and mutation bursts. These, in turn, facilitate the spread of stable ARGs via mobile genetic elements (blue box). The cycle feeds back into clinical settings, driving treatment failure and further drug use (dashed arrow). Interruption of this loop requires coordinated interventions targeting genetic mechanisms, ecological reservoirs, and human behaviour. Solid arrows represent primary pathways.

2.2. Biochemical Mechanisms of Resistance

Resistance manifests through four primary biochemical strategies:

- Enzymatic inactivation: β-lactamases—including extended-spectrum β-lactamases (ESBLs), AmpC, and carbapenemases such as KPC, NDM, and OXA-48—hydrolyse the β-lactam ring [27]. Aminoglycoside-modifying enzymes (acetyl-, adenyl-, and phospho-transferases) disable kanamycin, gentamicin, and amikacin, while the flavin-dependent monooxygenase *tet(X4/X5)* inactivates last-line tigecycline and has already disseminated in livestock plasmids across China and Europe [28,29].
- Target modification: Altered penicillin-binding proteins (e.g., PBP2x in Streptococcus pneumoniae and PBP2a encoded by *mecA* in MRSA) reduce β-lactam efficacy [30]. erm-encoded rRNA methyltransferases block macrolide, lincosamide, and streptogramin binding [31], while acquisition of *vanA* and *vanB* operons replaces the D-Ala-D-Ala terminus with D-Ala-D-Lac in peptidoglycan precursors, conferring vancomycin resistance [32,33].
- Reduced permeability: Loss or truncation of porins (e.g., OmpK35/OmpK36 in *Klebsiella*) and increased capsule thickness restrict β-lactam entry. Over-expression of chromosomal efflux systems such as AcrAB-TolC and MexAB-OprM further halves intracellular drug concentrations [34–36].

- Active efflux: Five superfamilies—ABC, MFS, SMR, MATE, and RND—export a wide array of antibiotics, dyes, and disinfectants [37]. Among these, RND pumps are particularly potent in Gram-negative pathogens and frequently co-select for heavy-metal tolerance [38].

2.3. . Ecological and Physiological Drivers

Ecological contexts and stress responses also shape AMR trajectories. Sub-inhibitory antibiotic gradients in wastewater, soils, and aerosols serve as evolutionary proving grounds, enriching mutants that would be out-competed at therapeutic concentrations. Co-selection by metals and biocides further sustains resistance; for example, copper, zinc, and quaternary ammonium compounds share determinants such as class 1 integrons, meaning that even stringent antibiotic bans may not reverse ARG prevalence in feedlots or sludge [39,40]. Additionally, oxidative stress and SOS responses triggered by fluoroquinolones elevate mutation rates and promote integron cassette rearrangements, accelerating ARG diversification [41,42].

2.4. One-Health Connectivity

High-resolution genomic studies now trace specific ARGs across clinical, animal, and environmental reservoirs. For instance, an *IncFII blaNDM-5* plasmid has been reported both in neonatal sepsis isolates from Pakistan [43] and in companion-dog faeces in Belgium (2023) [44]. In the Netherlands, hospital, household, and companion-dog *Escherichia coli* ST131 isolates differed by fewer than five single-nucleotide polymorphisms (ANI ≥ 99.85%) and all carried an *IncFII blaCTX-M-15* plasmid [45]. In Spain, yellow-legged gulls were found to harbour an *IncK blaCTX-M-1* plasmid identical to those in pig-farm isolates located far inland [46]. Atmospheric transport also plays a role: spring storms in northern China were shown to move *intI1* and *sul1* more than 100 km down-wind [47]. Together, these findings highlight the tight One Health connectivity of AMR and underscore the need for surveillance strategies that transcend traditional sectoral boundaries.

3. Public-Health and Economic Impact of Antimicrobial Resistance

AMR begins with therapeutic failure at the bedside, but its repercussions radiate through health systems, economies, food chains, and social structures. This part tracks that escalation—patient, health-system, macroeconomy, food & trade, society, pandemic amplifier—and closes by weighing the return on investment (ROI) of intervention packages.

AMR now claims 1.35 million lives a year [3] and triggers cascading costs: 2.6 million excess EU bed-days [48], a projected 3.8% GDP drag, crop losses, rejected exports and deepening household debt. Viral pandemics compound these effects, while climate warming accelerates environmental selection [49]. OECD and World Bank models converge: early, low-tech interventions yield a 1:6 pay-off [5,50], whereas delay increases costs exponentially. Strategic layering—prescription control, water-sanitation-hygiene (WASH), vaccines as the foundation, rapid diagnostics and economic pull incentives as safeguards—offers the most resilient defence [2]. Figure 2 below provides a global snapshot of resistance levels across eight major bacterial pathogens and multiple antibiotics, using surveillance data compiled up to 2023. The heatmap illustrates stark geographic and species-specific variation in resistance burdens, with *E. coli, K. pneumoniae* and *P. aeruginosa* exhibiting multi-drug resistance in several high-burden countries. This rising resistance landscape directly underpins the clinical toll and downstream economic impacts outlined above.

3.1. Clinical Toll—Deaths, Disability, Mental-Health Sequelae

Clinical outcomes are consistently worse when resistant pathogens are involved: in the PANORAMA multinational cohort, carbapenem-resistant Klebsiella pneumoniae bloodstream infections carried a mortality of 27%, compared with 11% for susceptible strains [51]. Beyond mortality, sepsis survivorship itself imposes major long-term burdens. A landmark U.S. cohort study showed that 30–50% of severe sepsis survivors developed persistent cognitive or functional impairment one year after discharge [52]. A subsequent review confirmed that approximately one-third to one-half of survivors experience lasting disability or reduced quality of life [53]. Mental-health sequelae further widen this footprint: a UK-wide prospective study of 4943 ICU survivors found that 18% met DSM-5 criteria for post-traumatic stress disorder at six months [54]. Collectively, these findings demonstrate AMR's "triple burden": excess deaths, lasting disability, and psychological trauma, all of which demand life-course care and rehabilitation planning.

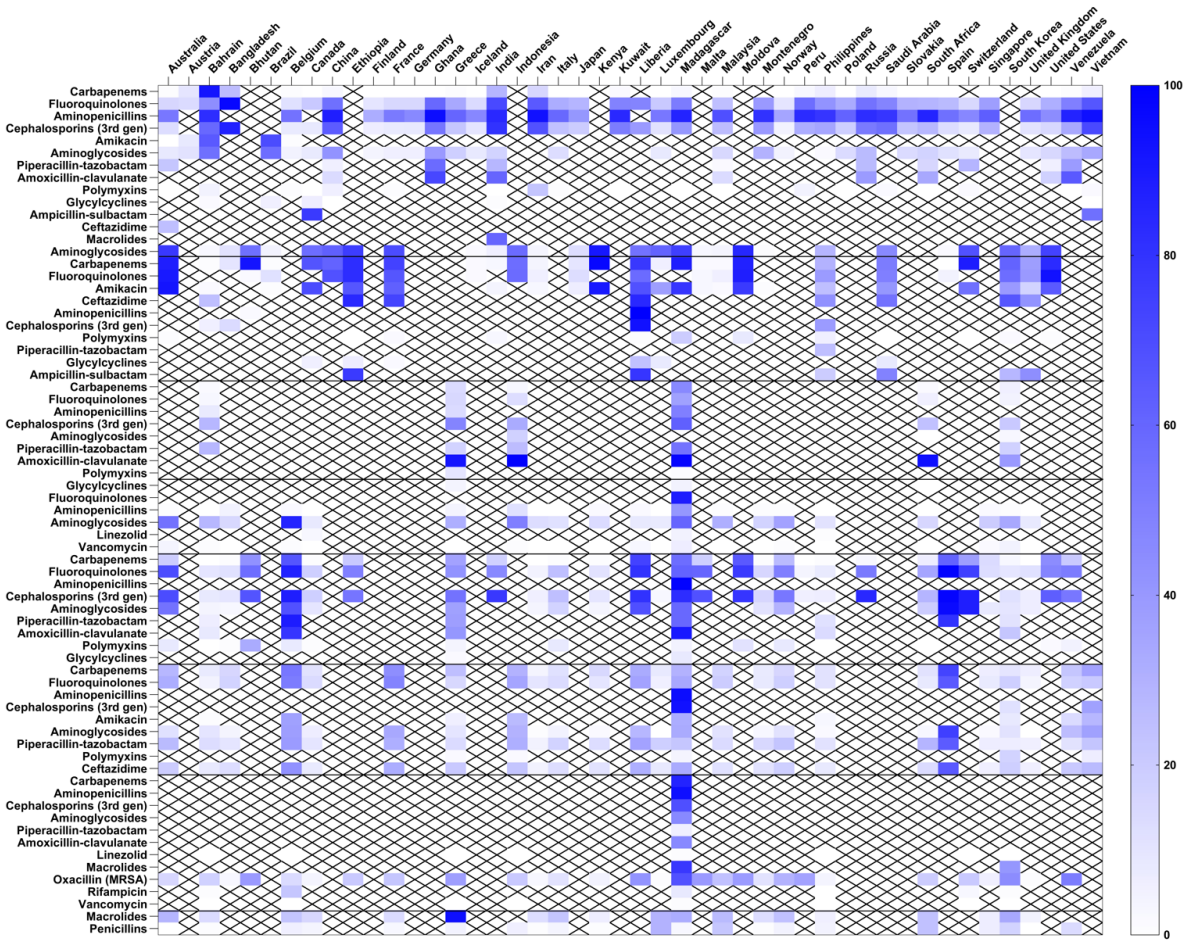

Figure 2. Heatmap showing the percentage of resistant isolates for eight major bacterial pathogens across multiple antibiotic classes and countries. Colour scale indicates the percentage of resistant isolates (% resistance). Cells marked with "X" indicate no available data. Data were compiled from WHO GLASS and ResistanceMap (accessed 2023).

3.2. Health-System Overload—Beds, Staff, Supply and Innovation

In the European Union/European Economic Area (EU/EEA), about 4.3 million patients acquire a healthcare-associated infection (HAI) each year, with resistant bacteria responsible for a substantial proportion of this burden [49]. In the United States, multidrug-resistant infections significantly increase mortality, length of stay, and hospital costs [55]. Meanwhile, low profitability and repeated market exit of small and medium enterprises (SMEs) continue to undermine the antibiotic pipeline, exacerbated by recurrent shortages of essential agents [56]. Together, these pressures deplete bed capacity, destabilise supply chains, and deter innovation, underscoring the urgent need for coordinated prevention and incentive strategies.

3.4. Food System & Trade—Hidden Nutritional Costs

AMR also undermines food systems and trade. In the US beef industry, *Pasteurella multocida* is a key pathogen within the bovine respiratory disease complex, a leading cause of illness and death in feedlot cattle. Estimates place the annual economic losses from this disease at USD 800–900 million due to mortality, reduced feed efficiency, and treatment costs [57,58]. In Vietnam's Mekong Delta, a survey of 45 small-scale freshwater aquaculture farmers found that tilapia growers often used florfenicol, and commonly switched drugs if initial treatments failed—an approach associated with concerns over residue breaches and increased [59]. In Europe, some retailers enforce pesticide and antimicrobial residue standards stricter than official maximum residue limits, further constraining producers. Although transferable ARGs such as *qnrS* have been detected in reclaimed irrigation water systems, no published evidence yet shows these genes on the surfaces of irrigated fruits or vegetables [60]. These cases highlight how resistance and residue issues threaten productivity, food safety, brand reputation, and export revenues across livestock, aquaculture, and crop sectors.

3.5. Societal Costs—Poverty Loops & Gender Gaps

Drug-resistant infections exacerbate household hardship and widen gender inequalities. In Bangladesh, analysis of the 2016 Household Income & Expenditure Survey (46,076 households) showed that 24.6% of families faced catastrophic health spending at the 10% threshold, and medicines accounted for about 70% of all out-of-pocket costs [61,62]. A cost-of-illness study of paediatric RSV hospitalisations in Dhaka found that one admission absorbed a median 32% of monthly income and forced just over half of carers to borrow or sell assets [63]. The 2024 Bangladesh Time-Use Survey further revealed that women perform seven times more unpaid domestic and care work than men, limiting their participation in paid employment. Taken together, these findings show how AMR—by driving up treatment costs and prolonging illness—pushes households into financial distress and places a disproportionate care burden on women, reinforcing cycles of poverty and limiting timely access to effective treatment [64].

3.6. Pandemic Amplifier—AMR in a Viral Outbreak World

Viral pandemics can amplify AMR, producing a lethal synergy that imperils surgery, oncology and transplant medicine. Meta-analyses show that roughly 75% of hospitalised COVID-19 patients—and up to 86% in ICUs—received empiric antibiotics during the first pandemic wave. During the COVID-19 pandemic, the prevalence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) surged in Italian hospitals. In a study conducted across Southern Italy, CRAB accounted for 80.8% of all *A. baumannii* isolates during 2020, highlighting a marked increase compared to pre-pandemic levels [65–67].

3.7. Cost–Benefit Landscape and Policy Levers

Foundational interventions–including safe water, routine vaccination and strict prescribing limits–yield the best economic returns, while rapid diagnostics and novel antibiotics serve mainly as "tail-risk insurance." An OECD micro-simulation covering 34 high-income countries estimates that fully rolling out a core package of stewardship, prescription caps, enhanced hand hygiene and environmental cleaning for 10 years would cost about USD 20 billion, avert roughly 17,000 AMR-related deaths and generate a return on investment of about 5:1 [68]. Adding one-hour bedside PCR testing for sepsis triage raised programme costs to USD 33 billion, with only marginal health gains, reducing ROI to about 2:1 [69]. However, expanding the package to include WASH upgrades and universal infant pneumococcal-conjugate (PCV) and rotavirus vaccination would cost around USD 41 billion but could prevent approximately 750,000 deaths annually—largely from diarrhoeal and pneumococcal disease in addition to AMR—and the aggregate ROI increases to about 6:1 [70].

4. Environmental Reservoirs and Dissemination Pathways of AMR

4.1. Fresh & Drinking Water

Urban rivers, lakes and even treated tap water now harbour a "core" resistome dominated by *sul1, intI1, bla_TEM, tet(W)* and *qnrS* [71]: 74% of 101 nations had at least one multi-drug-resistant (MDR) gene detected in municipal sewage samples [72]. Routine metagenomics along a full-scale drinking-water plant in Shanghai showed a 1.7-log10 reduction in total ARG copies, yet *bla_CTX-M* persisted at 10^2 copies/mL in finished water [73]. Extreme rainfall exacerbates contamination: combined sewer overflows can raise riverine ARG loads three- to ten-fold, while agricultural tile drains contribute tetracycline and macrolide genes. In peri-urban aquifers, groundwater draw-down concentrates ARG-bearing bacteria in wells [74,75].

4.2. Wastewater and Wastewater Treatment Plants

WWTPs are major ARG hotspots. Hundreds of ARG subtypes spanning 21 drug classes have been detected across aerosols, sludge, and water at Chinese WWTPs [76]. Disinfection removes viable bacteria, yet free DNA maintains up to 40% of influent ARG copy numbers in UV-treated effluent [77]. A harmonised qPCR survey of 15 full-scale municipal WWTPs (eight in China, seven in the EU) found influent sewage containing 10^4–10^7 ARG copies/mL. Abundance rose to ~1.0×10^8 copies/mL in aerated activated-sludge basins, where biomass flocs selectively enriched resistant bacteria. Secondary clarification reduced loads to ~2.51×10^6 copies/mL, while final disinfection (chlorination or UV) produced effluent averaging 7.49×10^5 copies/mL. These findings indicate that conventional tertiary treatment substantially reduces but does not eradicate plasmid-borne β-lactamase and sulfonamide genes [78].

4.3. Livestock Farms and Agricultural Soils

Global antimicrobial use in food-producing animals was ≈99,500 tonnes in 2020 and, without policy change, is projected to rise by ~8% by 2030, reaching ~107,000 tonnes [79,80]. Usage intensity varies sharply between species: global averages indicate that sheep and pigs receive the highest antibiotic inputs (243 and 173 mg/kg of meat, respectively), while cattle and poultry require far less (60 and 35 mg/kg) (Figure 3). Heavy use—especially where oversight is weak—intensifies selection and spread of resistance along the farm–environment continuum. For example, surveys of Chinese broiler operations report that 60–70% of Escherichia coli isolates carry extended-spectrum β-lactamase genes, with *blaCTX-M-55* among the dominant alleles [81,82]. In Catalonia, a plot-scale study showed that lettuce irrigated with river water picked up the soil's ARG hierarchy (*sul1* > *tetM* > *intI1*), although gene copies on leaves were one to two orders of magnitude lower than in soil [83,84]. Manure-based fertilisers further increased risk: global soil surveys and field experiments indicate a two- to three-fold rise in co-located antibiotic- and metal-resistance genes after organic-manure application, with *sul1* frequently embedded in *Tn21*-like transposons [85,86]. These findings underscore how intensive drug use, contaminated irrigation water and manure recycling together magnify the environmental footprint of antimicrobial resistance.

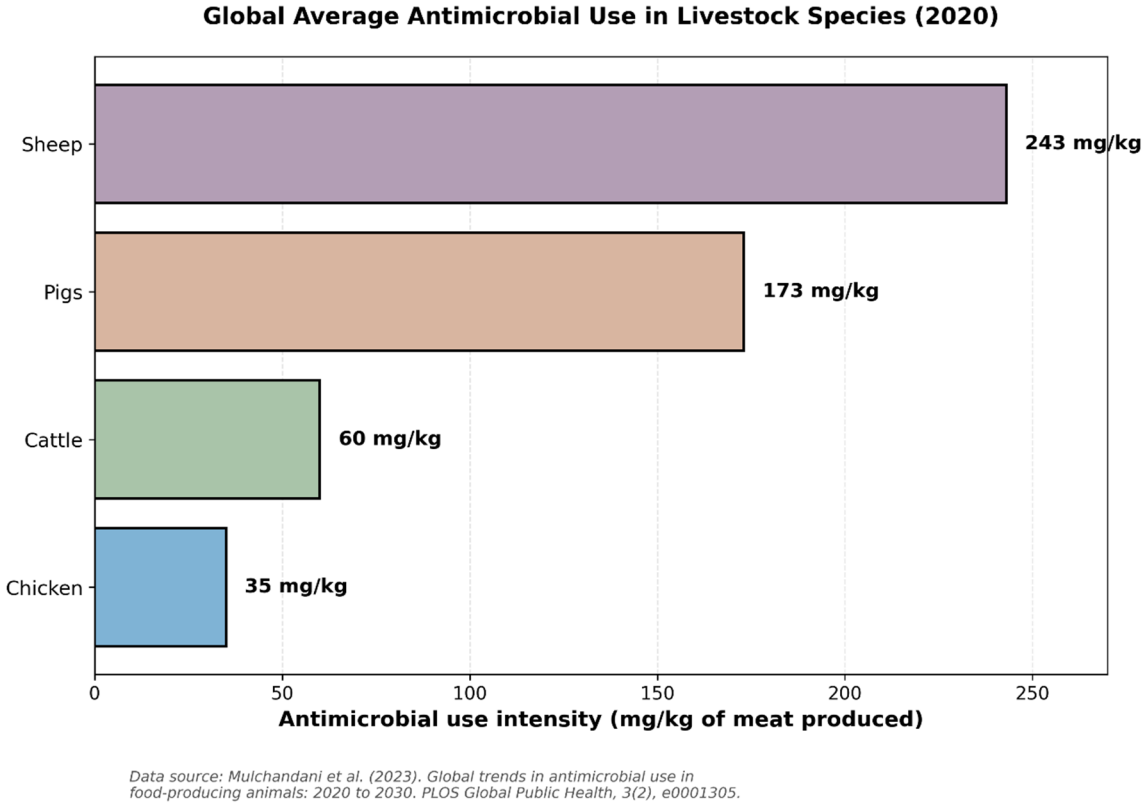

Figure 3. Global average antimicrobial use in livestock species in 2020, expressed as milligrams of active ingredient per kilogram of meat produced. Sheep (243 mg/kg) and pigs (173 mg/kg) show substantially higher antibiotic intensity compared to cattle (60 mg/kg) and chicken (35 mg/kg). Data source: Mulchandani, R., et al. (2023). Global trends in antimicrobial use in food-producing animals: 2020 to 2030. PLOS Global Public Health, 3(2), e0001305. https://doi.org/10.1371/journal.pgph.0001305 [87,88].

4.4. Landfills and Pharmaceutical-Production Effluent

Even stringent bans on growth-promotion antibiotics cannot reverse ARG prevalence if pharmaceutical hotspots and landfill leakage remain unchecked. Leachates from municipal landfills can exceed river concentrations of certain antibiotics by two orders of magnitude: Sulfonamides, quinolones and macrolides were found at 0.7–9.4 μg/L in leachate from 12 European sites [89]. Landfill soils are equally critical, hosting multidrug-resistant genes (MDRGs) at 1.9×10^4 copies/g, a 100× enrichment over adjacent sediments [88]. The most extreme contamination occurs near drug manufacturing hubs, for example, ciprofloxacin concentrations in effluent streams near Hyderabad, India, reached 14 mg/L, the highest recorded globally, attributed to active pharmaceutical ingredient production [90].

4.5. Airborne Dissemination

ARGs can adsorb onto fine particulate matter (PM2.5/PM10) and bioaerosols, enabling transport over kilometres. A meta-analysis of 29 studies revealed atmospheric ARG concentrations ranging from 10^2 to 10^5 copies/m³, with *sul1* (sulfonamide resistance) and *ermB* (macrolide resistance) being the most prevalent [91]. At a Portuguese WWTP, aminoglycoside resistance genes (*aadA, aph(3')-IIIa*) and quinolone-resistance genes (*qnrS, oqxB*) showed enrichment factors of 8- to 12-fold from influent water to aeration-tank aerosols, whereas glycopeptide genes (*vanA*, *vanB*) were near or below detection—linking aerosolisation propensity to the efflux-pump genotypes of host bacteria [92]. In eastern Canada, downwind sampling of mixed crop–livestock farms detected *mcr-1* in 6 of 10 high-volume air samples (~11%), despite a decade-long ban on colistin feed additives, suggesting long-range or legacy transport [93]. Meteorological conditions also modulate ARG burdens: high humidity (>70%) and low wind speed (<2 m/s) favour retention in near-source plumes, whereas semi-arid regions (<40% relative humidity) report the lowest concentrations [94].

5. Analytical Toolbox for Tracking Environmental ARGs

The matrix positions each mainstream environmental-ARG assay along a trade-off curve that pits detection limit against information depth. Selective culture, antimicrobial-susceptibility testing (AST), and single- or multiplex PCR achieve detection limits of 10–10^2 CFU/mL or 10–100 gene copies at minimal cost, but they miss viable-but-non-culturable (VBNC) cells and offer limited host context. High-throughput microfluidic qPCR arrays excel when sample volume is scarce (e.g., aerosol filters) and >300 ARGs must be screened simultaneously, though chip costs and cross-hybridisation among homologous genes necessitate rigorous quality control [95,96]. Shotgun metagenomics, particularly when combined with long-read and Hi-C techniques, resolves the full ARG–mobile element–host triad in a single workflow and can detect alleles accounting for <0.01% of total reads [97–99]. The trade-off is greater data volume, higher reagent cost, a steeper bioinformatics learning curve.

5.1. Selective Culture and Phenotypic Antimicrobial Susceptibility Testing

Standardised disk-diffusion and broth microdilution assays (CLSI M100, 34th ed., 2024) provide minimum-inhibitory-concentration (MIC) values for isolated colonies and remain the global benchmark for phenotypic antimicrobial-susceptibility testing [100]. In environmental monitoring, modified-mTEC agar (EPA Method 1603) reliably enumerates *Escherichia coli*: an inter-laboratory validation of 20 U.S. labs reported mean recoveries of 64–96% within 24 h across spiked water and wastewater matrices [101]. Automation platforms such as VITEK-2 accelerate throughput and deliver MICs directly from ID/AST cards but still require a 0.5 McFarland suspension (~1.5×10^8 CFU mL^{-1}) prepared from pure colonies [102]. Culture-based methods remain indispensable for risk assessment, as they link genotype to clinically relevant phenotypes, but they overlook VBNC cells and the majority of uncultured taxa.

5.2. Quantitative PCR and Digital PCR

Quantitative PCR (qPCR, SYBR Green or TaqMan probe-based) reliably quantifies ARGs at concentrations as low as 10 copies per reaction in purified DNA extracts [103]. Chen et al. (2019) documented a 1.7-$\log_{10}$ reduction of *sul1* from raw influent to tap water in a drinking-water treatment plant, highlighting the efficacy of purification processes. For absolute quantification in complex matrices, droplet digital PCR (dPCR) partitions samples into ~20,000 nanodroplets, achieving single-copy sensitivity and high inhibitor resistance [104]. Droplet-digital PCR (ddPCR) partitions each reaction into ≈20,000 nanolitre droplets, enabling absolute quantification with single-copy sensitivity while tolerating potent PCR inhibitors. Cavé et al. (2016) showed that ddPCR still quantified the *sul1* gene in manure extracts at 1.6 copies/reaction, whereas qPCR lost sensitivity below 15 copies because of matrix inhibition [105]. Micro-fluidic dPCR likewise detected *intI1* in humic-rich marine sediments at <5 copies/reaction without sample dilution [106]. Although consumables are costlier than qPCR (≈ USD 5–6 vs. 1–2 per reaction) and the droplet reader adds capital expense, ddPCR remains the method of choice for inhibitor-laden samples such as sludge, digested manure or chlorinated effluent, as first emphasised by Hindson et al. (2011) [107].

5.3. High-Throughput qPCR (SmartChip, WaferGen)

Nanolitre arrays allow 384–960 primer sets and 48–96 samples per chip. Su et al. profiled 384 ARGs across 30 WWTP aerosols, detecting 312 targets at ~10 copies/reaction [108]. However, cross-hybridisation can inflate low-abundance calls, necessitating Ct < 28 thresholds and melt-curve checks.

5.4. Shotgun, Long-Read and Synthetic-Linkage Metagenomics

Short-read (Illumina) metagenomes capture resistome diversity and allow normalisation to cell abundance (RPKM). The Global Sewage Resistome Survey (101 countries) hinged on this approach [72]. Long-read sequencing technologies (e.g., Oxford Nanopore and PacBio HiFi) enable complete assembly of ARGs–carrying plasmids into single contigs. Recently, Ma et al. (2023) applied Nanopore sequencing to isolates from pig farms in China and successfully reconstructed multiple *IncHI1* plasmids exceeding 200 kb in size, each carrying *tet(X4)* and other resistance islands [109]. Similarly, Mohsin et al. (2021) combined Nanopore and Illumina sequencing to resolve a ~260 kb *IncHI1*-type *tet(X4)* plasmid from poultry and environmental samples in South Asia, demonstrating the utility of long-read platforms in resolving repetitive regions and revealing structural diversity among multidrug resistance plasmids [110].

New linkage approaches address the critical "who-has-what" question by connecting ARGs to their hosts. Hi-C proximity ligation physically couples plasmid or integron DNA with chromosomal DNA, enabling host assignment in complex communities; in wastewater, Stalder et al. recovered host information for ~70% of ARG-containing contigs [111]. The HAM-ART pipeline later streamlined this process [112]. Likewise, epicPCR (emulsion, paired-isolation and concatenation PCR) fuses an ARG marker with the 16S rRNA barcode of the same single cell. The original lake-water study retrieved species-level hosts for rare functional genes [113], while a long-read epicPCR upgrade raised resolution above 50% for clinically relevant ARGs in soil and manure microbiomes [114]. Shotgun and linkage libraries typically generate 20–100 gigabases of sequence data per sample, requiring dedicated pipelines such as ARGs-OAP v2.0 (HMM-based annotation against the SARG database) [115] and ResFinder 4.0 (genotype–phenotype prediction from assembled contigs) [116].

Per-sample costs, reconstructed from 2019–2024 audits and April 2025 catalogue prices, are summarised in Table 1, covering reagents, consumables, equipment, and labour.

Table 1. Estimated per-sample costs of major ARG-surveillance workflows (2024 USD), covering reagents, consumables, labour, and equipment amortisation. Representative cost sources span 2019–2025 catalogue prices and peer-reviewed audits.

Method	Single-Sample Cost	Representative Sources (2019–April 2025)
Selective culture + AST	≈12	Chromogenic plates (Thermo Fisher Brilliance series) Thermo Fisher; AST discs 2024 price list (Bioanalyse) bioanalyse.com; Bruker MBT Biotarget-96 consumable page bruker.com; CLSI M100 workflow times (33rd ed., 2023)
Single-plex PCR	≈6	DreamTaq price (Thermo Fisher catalogue) Thermo Fisher; PCR cost audit (Sherry 2019, Microbial Genomics)
qPCR (SYBR/TaqMan)/ddPCR	≈24	Bio-Rad QX200 consumables price sheet bio-rad.com; Karkman 2019 workflow description (Water Research)
SmartChip/WaferGen HT-qPCR array	≈60	Takara Bio SmartChip ND (Takara Bio) takarabio.com; WaferGen chip price report (GenomeWeb) genomeweb.com;
Short-read shotgun (NovaSeq SP)	≈155	NovaSeq SP flow-cell price list (NC State Genomic Sci. Lab, May 2024) Office of Research and Innovation;
Long-read + Hi-C metagenomics	≈225	ONT flow-cell store price store.nanoporetech.com; ProxiMeta kit list price Phase Genomics;
High-throughput culturomics	≈85	Bruker Biotarget data bruker.com; Illumina MiniSeq service menu (NCSU 2024) Office of Research and Innovation

6. Policy Roadmap and Research Agenda

6.1. Real-Time, Risk-Weighted Surveillance

Dutch nationwide sewage studies already track carbapenemase-producing *Enterobacterales*, and machine-learning prototypes for gene-trend prediction are in development, though peer-reviewed accuracy metrics are still pending [117,118]. MIQE 2.0 (2024) now mandates essential metadata for qPCR and dPCR assays, and an AMR-specific addendum has been proposed for 2026. Meanwhile, the Genomic Standards Consortium is considering an "MIMAG-AMR" extension to its metagenome-assembled genome (MAG) checklist [119] Portable field-ready "lab-in-a-backpack" systems built around Bento Lab, MinION, and hand-held dPCR units are undergoing international field trials, with full evaluation expected in 2025.

6.2. Behavioural and Stewardship Interventions

Primary care interventions demonstrate impact. In Australia, the "Nudge-vs.-Superbugs" randomised trial mailed peer-comparison letters to high-prescribing general practitioners, reducing total antibiotic prescriptions by 12% within six months [120]. In livestock, Denmark's "Yellow Card" threshold system, launched in 2009, reduced antimicrobial use in pig herds by 25% within two years without compromising productivity [121]. Community-level studies in Bangladesh further show that social-marketing messages can reduce non-prescription antibiotic demand, though effect sizes still require validation in controlled trials [122].

6.3. Engineered Controls at High-Risk Sites

Technological interventions can reduce environmental burdens. Municipal WWTPs that retrofit membrane bioreactors with ozonation achieve 0.8–1.2 $\log_{10}$ reductions in *intI1* and *sul1* [123]. In hospital wards, portable HEPA units equipped with UV-C recirculation lamps cut airborne MRSA by at least two orders of magnitude [124]. For pharmaceutical effluent, NF90 nanofiltration eliminates >99% of ciprofloxacin and enrofloxacin residues [125,126].

Key research needs for 2024–2030 include:

1. External validation of wastewater-based ARG forecasts against clinical incidence across multiple jurisdictions.
2. Costed implementation models for CRISPR strip screening at community water points.
3. Standardised life-cycle assessment (LCA) methodologies to compare advanced WWTP upgrades on a cost-per-DALY basis.
4. Impact evaluations of bundled behavioural nudges plus point-of-care diagnostics in both human and veterinary sectors.

7. Conclusions

AMR is erasing a century of medical gains, yet an integrated, data-driven response can still bend the curve. Genome sequencing, metagenomics and long-read platforms now track resistance genes with single-gene resolution, while big-data pipelines and AI deliver real-time predictive analytics. Deployed through multi-level surveillance networks and open data-sharing, these tools flag hotspots, guide local prescribing, verify banned-drug compliance and steer next-generation antibiotic discovery.

Environments are not passive sinks; wastewater, soils and aerosols form dynamic bioreactors where sub-MIC antibiotic gradients enrich resistant mutants. Treatment failures then trigger heavier prescribing, closing a vicious loop that most users never see. Future research must also quantify downstream health effects—e.g., whether communities near ARG hotspots carry higher loads of resistant commensals—and feed those metrics into clinical risk models.

Policy architecture is emerging. Combining predictive surveillance, risk-based regulation, economic pull incentives and behaviour-change programmes under a One Health banner could plausibly halve AMR deaths within a decade. Remaining gaps—harmonised protocols, data integrity, privacy and cross-border trust—demand sustained investment and coordinated commitment from governments, industry, healthcare systems and civil society. The science is mature; the task now is to deploy, monitor and refine these interventions before the clock rewinds further.

Author Contributions

N.Z.: conceptualization, methodology, data curation, writing—original draft preparation, visualization, investigation; K.V.T.: supervision, resources, writing—review & editing; J.L.: formal analysis, validation, writing—review & editing; J.W.O.: supervision, funding acquisition, project administration, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

Funding

N.Z. is supported by a China Scholarship Council - University of Queensland (CSC-UQ) Research Training Program Scholarship. J.W.O. is supported by a National Health and Medical Research Council (NHMRC) Investigator Grant (EL1 2009209). The Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, acknowledges financial support from Queensland Health, Australia. No specific funding was received for article processing charges.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Use of AI and AI-Assisted Technologies

The authors used Grammarly and ChatGPT to improve language quality. All content was reviewed and edited by the authors.

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