Bio Vet Innovator Magazine
Volume 2 (Issue 11) NOVEMBER 2025
OPEN ACCESS World AMR Awareness Week (WAAW) - 2025 POPULAR ARTICLE

Wastewater and the Gut Microbiome: The Emerging Bioreactors Driving the Next AMR Pandemic

Dr. Vaidehi Deorao Chandankar [1], Dr. Sonali D. Chandankar [2]
1. Department of Veterinary Microbiology, ICAR-Indian Veterinary Research Institute, Hebbal, Bengaluru- 560024, Karnataka, India
2. Assistant Manager, Poultry Diagnostics and Research Center, Pune-412201, Maharashtra, India
***Corresponding Author:** chandankarvaidehi@gmail.com*
DOI: https://doi.org/10.5281/zenodo.17951465

Received: November 27, 2025
Published: November 30, 2025

Abstract:

Wastewater is a silent incubator where antibiotics resistant bacteria (ARB), and resistance genes (ARGs) interact, exchange, and evolve into stronger threats. Human, animal, and hospital waste continuously release ARG-loaded microbes and antibiotic residues into wastewater systems, creating hotspots for resistance amplification. The gut microbiome further acts as a natural ARG factory, where commensals, pathogens, and environmental bacteria exchange genes through plasmids, integrons, transposons, and bacteriophages. Horizontal and vertical gene transfer, biofilm protection, and selective pressure from antibiotic residues accelerate the emergence of multidrug-resistant strains. Metagenomics has now emerged as a powerful tool to detect, track, and characterize these hidden resistomes directly from environmental samples. Understanding wastewater as a dynamic antimicrobial resistance (AMR) bioreactor is crucial to designing effective surveillance, containment, and microbiota-based interventions to prevent the next resistance pandemic.

Keywords: *AMR, Wastewater, Gut microbes, Antibiotics Resistant bacteria (ARBs), Antibiotics Resistance genes (ARGs), Horizontal gene transfer (HGT)*

Wastewater as Hidden AMR Hotspot:

Sewage is no longer just dirty water—it has now become a breeding place for antimicrobial resistance. Wastewater treatment plants (WWTPs) in cities act as major collection centres of antibiotics, resistant bacteria (ARBs), and resistance genes (ARGs) coming from households, hospitals, animal farms, and pharmaceutical industries, making them powerful hotspots for resistance development (Chen et al., 2021). In these systems, antibiotics and resistant bacteria come together, and with the help of plasmids and other mobile genetic elements, these genes exchange horizontally and vertically, accelerating their spread in the environment. A major contributor is the human gut, which naturally carries commensal

bacteria and resistance genes that continuously enter sewage through faeces, urine, sputum, bathing water, and even nasal mucus. Unexpectedly, even neonates, who never had antibiotic exposure, were found carrying resistant *E. coli*, proving how deeply rooted this problem is in human communities (Purohit, 2019). Further aggravating the issue, antibiotic residues from pharmaceutical manufacturing units, hospitals, and animal farms enter water bodies, creating strong selective pressure that helps resistant bacteria survive, evolve, and multiply. One study detected residues of 17 commonly used antibiotics, including quinolones, macrolides, sulfonamides, and cephalosporins from pharmaceutical WWTPs across seven European countries (Rodríguez-Beltrán et al.,2021). Hospital wastewater contains even higher levels of antibiotics and resistance genes 25% more antibiotics and up to 1.8-fold higher ARG concentrations than normal sewage, making it an even more potent hotspot. Additionally, livestock, particularly chickens, pigs, and cattle, release multiple classes of antibiotics and ARGs through manure, with over 284 different ARGs detected, highlighting animal waste as another major contributor. Since many antibiotics such as levofloxacin, erythromycin, and tetracyclines are excreted unchanged, they continue to exert selective pressure even after entering the sewage system, quietly fuelling the AMR crisis beneath our feet (Kulik et al., 2023).

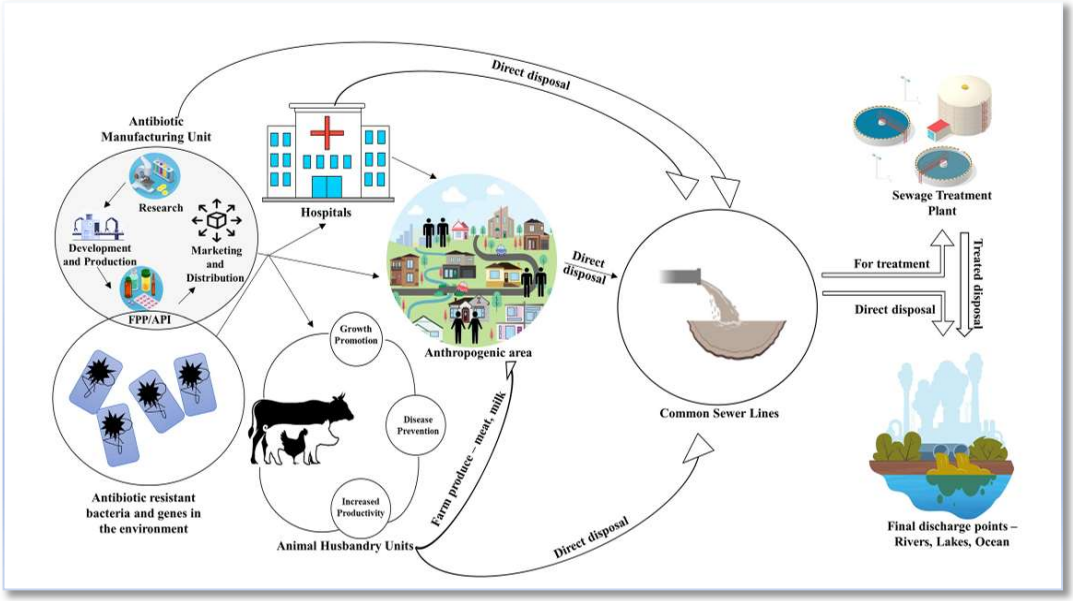

Figure 1: Dissemination of Antibiotic-Resistant Bacteria and Genes through Wastewater Pathways

Gut Microbiome: Natural Superbug Factory

The gut microbiome of animals, including livestock, pets, wildlife, and even humans, acts as a silent factory where antibiotic resistance genes (ARGs) are created, multiplied, and later released into the environment through feces and urine. The emergence and spread of ARGs through animal gut microbiomes play a pivotal role in antimicrobial resistance dissemination.

1. **Livestock Gut: A Hotspot for ARG Amplification and Global Spread**

Antibiotics in livestock and aquaculture are often used as growth promoters and prophylactics, leading to long-term low-dose exposure. This creates strong gut selective pressure, boosting ARG-carrying bacteria—e.g., amoxicillin and thiamphenicol increase beta-lactam and phenicol resistance genes in chickens. Companion animals like dogs and cats also serve as overlooked ARG reservoirs due to frequent antibiotic use and close contact with humans. Studies have shown a rise in multi-antibiotic resistance in their gut bacteria from 27% to 61% (Joosten et al.,2020). Even without antibiotic exposure, heavy metals such as copper and zinc used in animal feed can also promote resistance. Their genes often sit on the same plasmid as ARGs (co-resistance) or share protective mechanisms (cross-resistance), helping resistant bacteria multiply in the gut even without antibiotic use (Muurinen et al., 2021). Wildlife and migratory birds silently spread ARGs across regions. They acquire resistance from contaminated soil, water or prey, even in remote polar areas—and release it through fecal droppings. Captive animals (e.g., pandas, gorillas) show even higher ARG diversity due to antibiotic exposure and diet, making the animal gut a mobile engine of global AMR dissemination.

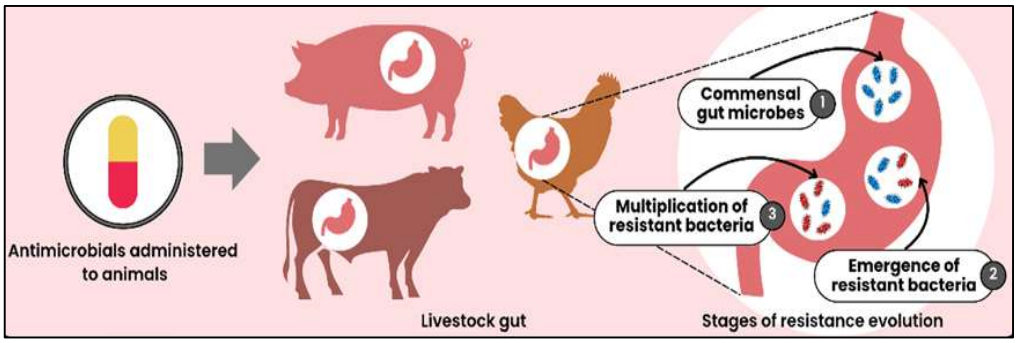

Figure 2: Pathway of Antimicrobial Resistance Evolution within Livestock Gastrointestinal Systems

2. Human gut

The consumption of antibiotics imposes selective pressure on bacterial communities inhabiting the human gut, skin, and blood (Drieux et al., 2016). Additionally, antimicrobial agents may also enter the human gut through secondary sources such as contaminated food and water. The human gut provides a favorable environment for horizontal gene transfer of ARGs via mobile genetic elements including plasmids, transposons, and integrons—along with genetic mutations and subsequent vertical gene transfer (Penders et al., 2013). Therefore, human populations act as major reservoirs of ARB and continuously contribute ARGs to sewage systems. The gut environment is characterized by mesophilic temperature (37 °C), nutrient-rich and anoxic conditions, high bile and salt concentrations, and high osmotic pressure. Most ARB in the human gut are obligate anaerobes (≈80%) and mesophilic (≈90%), while only a small proportion (≈11%) are facultative anaerobes (Hu et al., 2014).

3. Exchange of ARGs Between Gut Pathogens and Commensals

The gut functions as a dynamic hotspot for horizontal gene transfer (HGT), where resistance genes are frequently exchanged between Enterobacteriaceae and other gut bacteria, including both pathogens and

commensal flora. When the gut barrier is disturbed—due to infection by pathogens like *Vibrio cholerae*, *Salmonella spp.*, *Staphylococci*, or enteric viruses—microbial balance is disrupted, natural immunity weakens, and opportunities for ARG transfer increase (Stecher et al.,2012). Antibiotic exposure further accelerates this process by wiping out beneficial bacteria, particularly *E. coli* and anaerobes responsible for gut barrier integrity . Even a short macrolide course can alter the gut microbiome for years. Meanwhile, opportunistic organisms such as *Pseudomonas*, *Candida*, and *Enterococci* survive and proliferate, enhancing ARG exchange and promoting the emergence of multidrug-resistant strains.

4. Gut: Major Source of ARG Dissemination and Environmental Release

The gut acts as a hotspot for the emergence and dissemination of multidrug-resistant bacteria, especially in ICUs where bacterial load in the rectum reaches up to 10^8 CFU/g, making transmission via contaminated hands highly likely. Antimicrobial use disrupts gut microbiota, eliminating susceptible microbes and enriching resistant ones, leading to increased ARG shedding through feces and animal products. Oral administration exerts a stronger disturbance on gut flora than injectable routes. Moreover, mcr-1-carrying plasmids show high transfer rates among *E. coli* and can spread to *Pseudomonas aeruginosa* and Enterobacteriaceae, increasing the risk of resistance dissemination to human pathogens and accelerating AMR evolution.

How Wastewater Creates New Killer Resistance

Wastewater provides an ideal breeding ground for antimicrobial resistance (AMR). Bacteria here exhibit resistance either naturally (intrinsic) through mechanisms like efflux pumps, reduced membrane permeability, or absence of drug targets, or they acquire it through genetic changes. AMR spreads in wastewater mainly through:

- Horizontal gene transfer (HGT) using plasmids, transposons, and integrons
- Mutations that create new resistant strains
- Vertical transfer of these resistant traits to offspring

1. Horizontal Gene Transfer (HGT): The Genetic Highway for AMR Spread

Horizontal Gene Transfer is the transfer of genetic material between unrelated microbes, allowing quick acquisition of ARGs. It accelerates resistance spread far faster than mutation-based vertical transfer. It creates new, hybrid resistant strains by mixing genes from environmental, commensal, and pathogenic bacteria—fueling the global AMR crisis.

- Main Mechanisms of HGT includes-
  - Conjugation – direct cell-to-cell plasmid transfer (most common for ARGs).
  - Transformation – uptake of free DNA from the environment.
  - Transduction – bacteriophages transfer resistance genes.

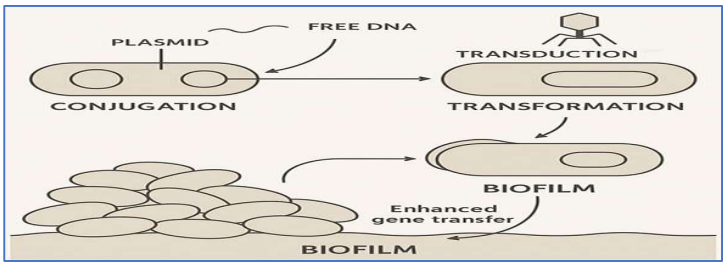

Figure 3: Horizontal Gene Transfer in Bacteria

- Mobile genetic elements (MGEs) like transposons, integrons, and gene cassettes facilitate ARG transfer, even between unrelated bacterial species.
- Conjugation via plasmids and MGEs (transposons, integrons, gene cassettes) is the dominant pathway for ARG dissemination.
- Conjugative plasmids have enabled the widespread global distribution of resistance genes for β-lactams, quinolones, sulphonamides, tetracyclines, and aminoglycosides (Huddleston 2014).
- Sun et al (2020) showed that people temporarily working in pig farms developed gut resistomes similar to full-time workers indicating direct environmental HGT.
- Biofilms present in wastewater form a protective matrix that shields bacteria from environmental stress and antibiotics, traps metals, biocides, and nutrients creating localized zones of high selective pressure, and promotes close cell contact, thereby accelerating conjugation and horizontal gene transfer (Luo et al., 2023).

2. **Vertical Gene Transfer (VGT)**

- Vertical gene transfer is the hereditary passage of genetic information, including mutation-derived resistance genes from parent to offspring through reproduction.
- In poultry, VGT naturally occurs across generations as chicks inherit gut microbes from hens through egg contact, reproductive tract transfer, cohabitation, and environmental microbial persistence (Shterzer et al., 2023).
- This direct inheritance helps maintain and transmit resistant bacteria and their genes within animal populations.

3. **Role of Bacteriophages & Gene Transfer Agents (GTAs)**

- Bacteriophages from poultry can transduce multiple resistance genes to *E. coli* (Shousha et al., 2015).
- GTAs resemble bacteriophages and facilitate gene transfer via transformation and transduction.

Impacts of AMR on One Health

1. Human Health Impact

Antibiotic resistance (ABR) reduces the effectiveness of treatments and increases infection severity, and the heavy use of antibiotics in animals further accelerates resistance in key human enteric pathogens like

E. coli, *Campylobacter*, *Enterococcus*, and *Salmonella* (O'Neill 2018). Fluoroquinolone and cephalosporin-resistant *Salmonella* is a leading public health problem in the world. Due to drug toxicity concerns, therapeutic options for vulnerable groups such as pregnant women and children are limited. The surveillance data of the WHO revealed a low fluoroquinolone resistance rate among non-typhoidal *Salmonella* in the European region (2–3%), a wide range in the Americas (0–96%), and a higher rate in the Eastern Mediterranean (up to 40–50%) (Organization WH, 2014). AMR continues to emerge in *Salmonella* strains and has been related to certain other life-threatening infections in humans.

2. Animal Health Impact

The major leading factors in methicillin-resistant S. aureus (MRSA) transmission in animals are the use of antibiotics in food, livestock, international trade of animals, and lapses in biosecurity within or between farms (Davis et al., 2017). Additionally, the exposure of pathogens to biocides such as antiseptics, disinfectants, and heavy metals in both environmental niches and animals may co-select for AMR. Some bacterial strains are regarded as gut commensals of humans and animals, while others may behave as donors of resistance genetic elements and opportunistic pathogens (Collignon, 2015). Among different water- and food-borne infections, *Campylobacter* infection is usually considered a self-limiting illness; however, fluoroquinolone-resistant *Campylobacter* severe infection has been reported due to prolonged antibiotic use. The detection of antibiotic residues in meat products as well as dairy and egg commodities poses public health risks and may lead to export bans for the affected region. Resistant infections in companion animals result in prolonged illness and repeated infections, which produce inadequate responses to treatments. The search for appropriate therapeutic options against MDR infections often proves difficult for veterinarians, who depend instead on costly medical alternatives that may have toxic side effects. The emotional costs and financial expenditures to pet owners demonstrate how AMR affects veterinary clinical practices along with emotional welfare.

3. Environmental Impact

In the environmental health situation, AMR is associated with the transmission of MDR pathogens and ARGs of public health concern (Banerji et al., 2019). Most drug resistance genes, pathogenic microbes, and antibiotics have environmental origins, such as soil and water. Evidence suggests that resistance to many antibiotics existed even before their clinical use; however, human activities have significantly contributed to the acceleration and expansion of the global resistome.With the massive annual production of antibiotics, environmental niches have become crucial reservoirs for the persistence and spread of AMR microbes. Inadequate sewage and pharmaceutical waste treatment leads to the discharge of antibiotics, resistant bacteria, and ARGs into natural water bodies, creating major hotspots for their transmission (Kraemer et al., 2019).Poor sanitation, globalized trade in food and animals, and international travel further facilitate the worldwide dissemination of resistant microbes.

To address environmental contributions to drug resistance, possible measures include risk assessment, environmental monitoring, and implementation of proper control strategies to reduce pollution from agricultural, industrial, and residential sources.

4. Economic & Global Impact

Numerous studies show that AMR infections lead to higher illness, deaths, and heavy healthcare costs. However, accurately measuring this burden is challenging due to differences in study methods, data quality, and reporting standards. According to the WHO, AMR is responsible for nearly 700,000 deaths annually worldwide, and this number is estimated to rise to 10 million by 2050 surpassing deaths caused by cancer and traffic accidents (Trotter et al., 2019). The Global Burden of Disease (GBD) collaborators further estimated that the overall health burden associated with AMR is potentially larger than that of HIV/AIDS and malaria combined.In the United States, the CDC reported that 2 million people are infected annually with antimicrobial-resistant microbes, with 23,000 resulting deaths. Since the disease burden is directly proportional to the economic burden, several studies have confirmed that AMR infections incur significantly higher healthcare costs compared to susceptible infections. Globally, developed countries may lose 1.1–3.8% of their GDP due to AMR by 2050, while Sub-Saharan African countries may face GDP losses between 0.1–2.5%, with even higher losses expected in low- and middle-income countries. The Organisation for Economic Co-operation and Development (OECD) estimated that the health systems of 33 countries spent around $3.5 billion annually in 2018 to address AMR-related complications, with $2 billion from the United States alone. These statistics clearly emphasize the urgent need to address this escalating global crisis.

Solutions to Combat the Hidden Antimicrobial Resistance Crisis

1. Strategies to Combat gut Antimicrobial resistance

The rising threat of gut antimicrobial resistance (AMR) is strongly linked to poor legislation, misuse of antibiotics, inadequate sanitation, lack of surveillance, and ineffective infection prevention. To counter this crisis, multi-level strategies are essential, including national measures, clinical interventions, personal actions, and microbiota-based solutions.

(I) National Strategies

a) AMR Surveillance and Early Detection- Integrated AMR surveillance systems across food, healthcare, sewage, and the environment—supported by metagenomic sequencing and real-time data networks—enable early detection, track ARG/ARB spread, and assess treatment efficiency, especially through wastewater resistome monitoring.

b) Reducing Environmental Dissemination- Enforce strict regulations on the discharge of untreated human, animal, and pharmaceutical waste, while promoting sanitation measures and sustainable approaches such as composting, probiotics in animal feed, and pyrolysis to convert fecal waste into biochar.

c) New Antibiotics & Vaccine Development- Promote non-antibiotic combinations, drug repurposing, and new molecule discovery, while encouraging vaccine use to reduce antibiotic dependence, lower hospital infections, and indirectly curb AMR.

(II) Clinical and Healthcare Strategies

a) *Rational Antibiotic Use*- Avoid unnecessary prescribing and self-medication, enforce antibiotic stewardship in both human and veterinary sectors, and prefer alternative administration routes (like injection or inhalation) over oral antibiotics to minimize gut resistome expansion.

b) Infection Control in Healthcare Settings- Enhance hygiene, disinfection, and sterilization; robust infection control and record-keeping restraint AMR spread.

(III) Personal Lifestyle & Hygiene Measures

a) *Boosting Human Immunity* - Adequate sleep, regular exercise, and balanced diet help maintain gut microbiome balance. Follow WHO Five Keys to Safer Food: clean, separate, cook properly, safe water/raw materials, and correct storage.

b) *Personal Hygiene & Environmental Cleanliness*- Handwashing, food safety, proper waste handling, and disinfectants reduce ARG transmission.

(IV) Microbiota-Mediated Protection

a) *Probiotic-Based Modulation*- Safe strains (e.g., Lactobacillus, Bifidobacterium) can help restore gut microbiota balance but must be screened for ARG carriage.

b) *Fecal Microbiota Transplantation (FMT)*- Transplanting healthy donor stool restores gut microbiome and reduces carriage of multidrug-resistant bacteria and genes. Proven effective in recurrent C. difficile cases and emerging as a tool to eliminate intestinal ARB (Millan et al., 2016).

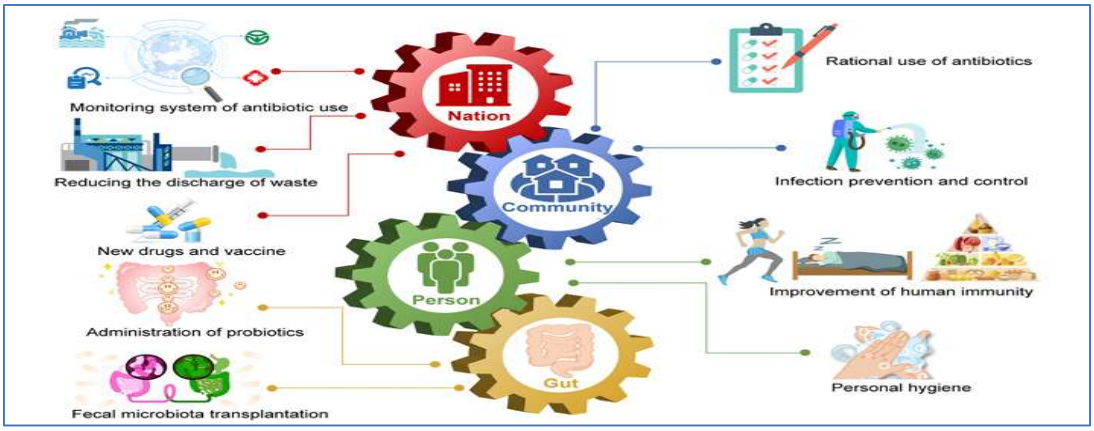

Figure 4: Strategies to combat gut antibiotic resistance from different levels

2.Metagenomics

Metagenomics is a DNA-based technique that allows direct analysis of microbial communities from environmental samples without culturing. It enables detection of resistant genes, mobile genetic elements, and virulence factors, helping in comprehensive resistome profiling (Forbes et al., 2017).

- Using shotgun sequencing and deep sequencing approaches, metagenomics identifies complete resistance gene clusters and reconstructs partial or whole genomes, making it indispensable for environmental antibiotic resistance analysis (Pal et al.,2016).
- Next Generation Sequencing (NGS) further allows comparison of sample-derived DNA with resistance gene databases like ARDB, SARG, CARD, and ResFinder using alignment-based homology to detect and quantify ARGs (Pillay et al.,2022).
- Functional metagenomics, combined with cloning and expression of environmental DNA in hosts like *E. coli*, helps detect ARGs based on their functional resistance patterns.

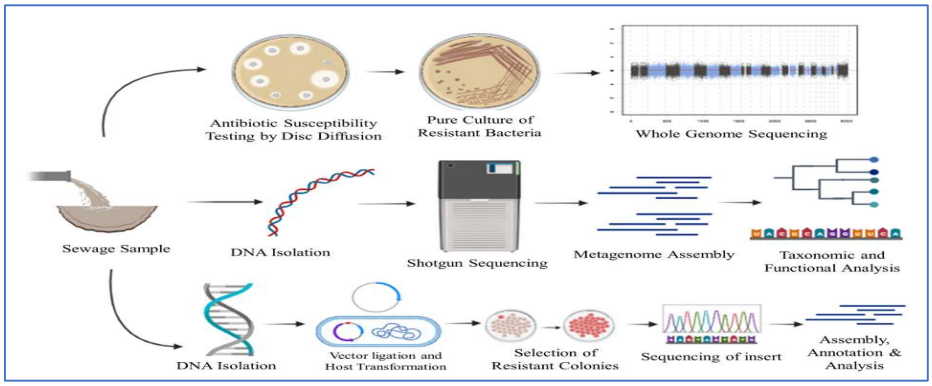

Figure 5: Metagenomics-Based AMR Detection Workflow

3. Alternatives To Antibiotics

I. Gene Editing Techniques- Gene editing tools (CRISPR, lysozyme, lysostaphin) directly target and inactivate mastitis-causing resistant pathogens. WGS-guided mutagenesis helps identify new targets. Though still under regulation, it is a promising antibiotic alternative.

II. Vaccines-Vaccines lower antibiotic dependence by preventing infections. Reverse vaccinology, using comparative and functional genomics, identifies conserved antigens (Brucella, E. coli, mastitis pathogens) for subunit vaccines. Transposon sequencing helps pinpoint essential virulence targets.

III. Anti-Virulence (AV) Inhibitors- AV inhibitors do not kill bacteria but block virulence mechanisms (e.g., toxin production, adherence, quorum sensing). Example: Virstatin inhibits *V. cholerae* virulence by downregulating ToxT regulator (Hung et al., 2005).

IV. Bacteriophage Therapy- Phages and engineered phage enzymes (endolysins, depolymerases) can specifically kill resistant bacteria in animals, aquaculture, and food safety (Patel et al., 2021). Before use, phages must be genome-sequenced to avoid transfer of ARGs or virulence genes. Tools like PHANOTATE, GeneMark, Glimmer help screen phage genomes safely

4. Technological Innovations in Wastewater and Manure Treatment

I. **Ozonation and Activated Carbon Adsorption**

- Eliminates up to 99.9% of ARGs (e.g., blaCTX-M-15)
- Cost-effective and widely implemented in wastewater treatment plants.

II. **Membrane Bioreactors (MBRs)**

- Combines biological and physical treatment, enhancing water quality and reducing ARG dissemination
- Reduces genes like mcr-1 (colistin resistance).

III. **Thermophilic Composting for Manure**

- Heating manure to 55°C for 7 days degrades tet (M) gene by ~99%.
- Limits transfer of resistance genes to soil and water.

IV. **Biochar Soil Amendment**

- Adding 5% biochar reduces extracellular DNA, limiting horizontal gene transfer.
- Enhances soil fertility and carbon sequestration; sustainable for organic waste management.

V. **Gamma-ray Irradiation**

- Reduces ARGs (tetO, tetA, blaTEM-1) by ~90.5% in wastewater effluents.
- Inactivates ARB and extracellular DNA, preventing environmental spread.

5. **Methods of Monitoring AMR**

I. Culture-Based Methods

- Culture-based methods on solid, semisolid, or broth media are gold standards for AMR detection, where antimicrobials help select resistant bacteria, and MIC (like broth microdilution for colistin) determines the minimum inhibitory concentration.
- Advantages: inexpensive, precise, convenient.
- Limitations: non-cultivable pathogens, false negatives, time-consuming, multiple steps, sensitive to specimen preservation.

II. Molecular Methods

- Detect pathogenic & commensal microbes genetically and monitors ARGs.
- Targets ARGs, 16s rRNA, integrons, insertion sequences, plasmid genes.
- PCR, qPCR, EpicPCR
- Metagenomics, Whole Genome Sequencing (WGS)
- Advantages: rapid, detects non-cultivable bacteria.
- Limitations: Detection doesn't ensure gene expression, and automated systems are costly and need regular updates

III. Mass Spectrometry

- MALDI-ToF MS: Protein profiling for bacterial ID & AMR.
- Rapid, accurate, large sample processing.
- Major brands: MALDI Biotyper® (Bruker), VITEK® MS (bioMérieux).
- Advantages- Rapid identification of bacteria and detection of some resistance markers with high

accuracy.

- Limitation- Cannot detect unknown or silent resistance genes and requires costly equipment and expertise

6. Surveillance of AMR

I. Clinical & National Surveillance

- Local & national AMR surveillance helps guide treatment protocols and intervention strategies.
- EARS-Net (1998, Europe): Collects routine antimicrobial susceptibility data from blood & CSF isolates of 7 key pathogens.
- GLASS (2015, WHO): Global surveillance; by 2020, 90 countries, reports data on up to 8 pathogens, strengthens global AMR monitoring.
- Advantages- Tracks resistance trends, Guides treatment, Supports public health decisions, Early warning system, Policy & global coordination
- Limitations: Small sample sizes, biased toward clinical settings, difficult in resource-poor areas, coordination issues in sampling and susceptibility testing.

II. Sewage-Based Surveillance

- Examines sewage inlets to treatment plants; already used in polio monitoring.
- Methods: Metagenomic sequencing (all ARGs) or qPCR (selected genes).
- Represents large urban population and generates resistome profile
- Advantages:
  - Captures community-level AMR beyond hospitals.
  - Easy to implement; inexpensive sampling and shipment.
  - Standardized sequencing & bioinformatics.
  - No ethical/legal issues (data not linked to individuals).
  - Monitors effects of interventions; useful where clinical diagnostics are limited.
- Limitations:
  - Does not link ARGs to specific species.
  - Sensitivity lower than isolate-based surveillance.

III. AI/Machine Learning(ML) in AMR Surveillance

- AMR surveillance and ML enable early detection, prediction, and clinical decision-making by linking resistance patterns with genomic data.

IV. Forecasting ARG Dissemination

- Using machine learning, spatial-temporal models, and surveillance data to predict how antibiotic resistance genes will spread across environments, populations, and regions.

V. Integration of AI with Computational Methods

- Combines AI, omics, ML, gene-sharing networks, MGE analysis to map ARG flow across ecological systems (e.g., livestock environments).

Conclusion

Antimicrobial resistance is quietly evolving beneath our feet in gutters, toilets, livestock farms, hospital drains, and even within our own gut. Wastewater serves as a perfect mixing chamber where antibiotics and microbes meet, mutate, and trade resistance genes, forming new and untreatable strains. The gut microbiome, especially in humans, livestock, and wildlife continuously supplies ARGs into sewage through fecal shedding, while selective pressure maintains their survival. These resistance traits later circulate back to humans via food, water, and environment, threatening One Health. Strong surveillance, wastewater monitoring, microbiome restoration, rational antibiotic use, and metagenomic tracking are essential to break this cycle. If ignored, sewage today may become the birthplace of tomorrow's untreatable superbug pandemic.

References

Banerji, A., Jahne, M., Herrmann, M., Brinkman, N. and Keely, S., 2019. Bringing community ecology to bear on the issue of antimicrobial resistance. *Frontiers in Microbiology*, 10.

Chen, Q., Li, D., Beiersmann, C., Neuhann, F., Moazen, B., Lu, G. and Müller, O., 2021. Risk factors for antibiotic resistance development in healthcare settings in China: a systematic review. *Epidemiology & Infection*, 149.

Collignon, P., 2015. Antibiotic resistance: are we all doomed?. *Internal medicine journal*, 45(11).

Davis, M.F., Rankin, S.C., Schurer, J.M., Cole, S., Conti, L., Rabinowitz, P., Gray, G., Kahn, L., Machalaba, C., Mazet, J. and Pappaioanou, M., 2017. Checklist for one health epidemiological reporting of evidence (COHERE). *One Health*, 4.

Drieux, L., Haenn, S., Moulin, L. and Jarlier, V., 2016. Quantitative evaluation of extended-spectrum β-lactamase-producing Escherichia coli strains in the wastewater of a French teaching hospital and relation to patient strain. *Antimicrobial Resistance & Infection Control*, 5.

Dutil, L., Irwin, R., Finley, R., Ng, L.K., Avery, B., Boerlin, P., Bourgault, A.M., Cole, L., Daignault, D., Desruisseau, A. and Demczuk, W., 2010. Ceftiofur resistance in Salmonella enterica serovar Heidelberg from chicken meat and humans, Canada. *Emerging infectious diseases*, 16(1).

Forbes, J.D., Knox, N.C., Ronholm, J., Pagotto, F. and Reimer, A., 2017. Metagenomics: the next culture-independent game changer. *Frontiers in microbiology*, 8.

Hu, Y., Yang, X., Qin, J., Lu, N., Cheng, G., Wu, N., Pan, Y., Li, J., Zhu, L., Wang, X. and Meng, Z., 2013. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. *Nature communications*, 4.

Huddleston, J.R., 2014. Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. *Infection and drug resistance*.

Hung, D.T., Shakhnovich, E.A., Pierson, E. and Mekalanos, J.J., 2005. Small-molecule inhibitor of Vibrio cholerae virulence and intestinal colonization. *Science*, 310(5748).

Joosten, P., Ceccarelli, D., Odent, E., Sarrazin, S., Graveland, H., Van Gompel, L., Battisti, A., Caprioli, A., Franco, A., Wagenaar, J.A. and Mevius, D., 2020. Antimicrobial usage and resistance in companion animals: a cross-sectional study in three European countries. *Antibiotics*, 9(2).

Kulik, K., Lenart-Boroń, A. and Wyrzykowska, K., 2023. Impact of antibiotic pollution on the bacterial population within surface water with special focus on mountain rivers. *Water*, 15(5).

Luo, T., Dai, X., Wei, W., Xu, Q. and Ni, B.J., 2023. Microplastics enhance the prevalence of antibiotic resistance genes in anaerobic sludge digestion by enriching antibiotic-resistant bacteria in surface biofilm and facilitating the vertical and horizontal gene transfer. *Environmental science & technology*, 57(39).

Millan, B., Park, H., Hotte, N., Mathieu, O., Burguiere, P., Tompkins, T.A., Kao, D. and Madsen, K.L., 2016. Fecal microbial transplants reduce antibiotic-resistant genes in patients with recurrent Clostridium difficile infection. *Clinical infectious diseases*, 62(12).

Muurinen, J., Richert, J., Wickware, C.L., Richert, B. and Johnson, T.A., 2021. Swine growth promotion with antibiotics or alternatives can increase antibiotic resistance gene mobility potential. *Scientific reports*, 11(1).

NewReportCalls for Urgent Action to Avert Antimicrobial Resistance Crisis. Available online: https://www.who.int/news/ item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis (accessed on 6 May 2022).

O'Neill, J., 2016. Tackling drug-resistant infections globally: final report and recommendations.

Organization WH (2014a). Antimicrobial Resistance Global Report on Surveillance: 2014 Summary (World Health Organization).

Patel, D.R., Bhartiya, S.K., Kumar, R., Shukla, V.K. and Nath, G., 2021. Use of customized bacteriophages in the treatment of chronic nonhealing wounds: a prospective study. *The international journal of lower extremity wounds*, 20(1).

Penders, J., Stobberingh, E.E., Savelkoul, P.H. and Wolffs, P.F., 2013. The human microbiome as a reservoir of antimicrobial resistance. *Frontiers in microbiology*, 4.

Pillay, S., Calderón-Franco, D., Urhan, A. and Abeel, T., 2022. Metagenomic-based surveillance systems for antibiotic resistance in non-clinical settings. *Frontiers in Microbiology*, 13.

Rodríguez-Beltrán, J., DelaFuente, J., Leon-Sampedro, R., MacLean, R.C. and San Millan, A., 2021. Beyond horizontal gene transfer: the role of plasmids in bacterial evolution. *Nature Reviews Microbiology*, 19(6).

Shousha, A., Awaiwanont, N., Sofka, D., Smulders, F.J., Paulsen, P., Szostak, M.P., Humphrey, T. and Hilbert, F., 2015. Bacteriophages isolated from chicken meat and the horizontal transfer of antimicrobial resistance genes. *Applied and environmental microbiology*, 81(14).

Shterzer, N., Rothschild, N., Sbehat, Y., Dayan, J., Eytan, D., Uni, Z. and Mills, E., 2023. Vertical transmission of gut bacteria in commercial chickens is limited. *Animal microbiome*, 5(1).

Stecher, B., Denzler, R., Maier, L., Bernet, F., Sanders, M.J., Pickard, D.J., Barthel, M., Westendorf, A.M., Krogfelt, K.A., Walker, A.W. and Ackermann, M., 2012. Gut inflammation can boost horizontal gene transfer between pathogenic and commensal Enterobacteriaceae. *Proceedings of the National Academy of Sciences*, 109(4).

Sun, J., Liao, X.P., D'Souza, A.W., Boolchandani, M., Li, S.H., Cheng, K., Luis Martínez, J., Li, L., Feng, Y.J., Fang, L.X. and Huang, T., 2020. Environmental remodeling of human gut microbiota and antibiotic resistome in livestock farms. *Nature communications*, 11(1).

Trotter, A.J., Aydin, A., Strinden, M.J. and O'grady, J., 2019. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. *Current opinion in microbiology*, 51.