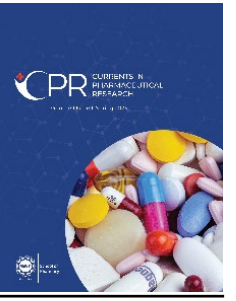
Currents in Pharmaceutical Research (CPR)
Volume 4 Issue 1, Spring 2026
ISSN$_{(P)}$: 3007-3235, ISSN$_{(E)}$: 3007-3243
Homepage: https://journals.umt.edu.pk/index.php/cpr



Title:	**A Detailed Review of Horizontal Gene Transfer as a Cause of Antibiotic Resistance: Environmental Vectors, Experimental Strategies for Intervention, and Molecular Mechanisms**
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DOI:	https://doi.org/10.32350/cpr.41.04
History:	Received: November 17, 2026, Revised: January 23, 2026, Accepted: March 02, 2026, Published: May 10, 2026
Citation:	Rasheed A, Raja K, Shoukat N, Kaleem S. A detailed review of horizontal gene transfer as a cause of antibiotic resistance: environmental vectors, experimental strategies for intervention, and molecular mechanisms. *Curr Pharm Res.* 2026;4(1):74-108. https://doi.org/10.32350/cpr.41.04
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Conflict of Interest:	Author(s) declared no conflict of interest

A publication of
The School of Pharmacy
University of Management and Technology, Lahore, Pakistan

A Detailed Review of Horizontal Gene Transfer as a Cause of Antibiotic Resistance: Environmental Vectors, Experimental Strategies for Intervention, and Molecular Mechanisms

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ABSTRACT

Horizontal Gene Transfer or HGT is an important mechanism leading to the global spread of antibiotic resistance, enabling bacteria to gain and share resistance determinants across taxonomic boundaries in environmental, clinical, and agricultural settings. This thorough analysis compares the corresponding contributions of the three main HGT pathways—conjugation, transformation, and transduction—to the spread of antimicrobial resistance (AMR) in terms of DNA transfer capacity, host range, and clinical frequency. Conjugation is the prominent mechanism in the clinical environment which is responsible for the inter-species dissemination of crucial resistance determinants, including *mcr-1* (mobilized colistin resistance), *blaNDM-1* (New Delhi metallo-β-lactamase-1], and *blaKPC* (*Klebsiella pneumoniae* carbapenemase), primarily via incompatibility group IncI2, IncX3, and IncF plasmids. Environmental storages—including wastewater treatment plants (WWTPs), manure-amended agricultural soils, and hospital biofilms—function as critical hotspots for antibiotic resistance gene (ARG) amplification and inter-kingdom gene exchange, where selective pressures from antibiotics, heavy metals, and biocides accelerates horizontal gene transfer and the evolution of multidrug-resistant microorganisms. Pakistan is nominated as an increased-risk region where the merging of inadequate diagnostic infrastructure, unregulated OTC antibiotic access, and poor wastewater management makes the environment that markedly speeds up HGT-mediated resistance. Innovative molecular intervention approaches, including DNase-functionalized nanocarriers, CRISPR-Cas9-based plasmid curing, and engineered bacteriophages, demonstrate significant preclinical promise but face extensive translational hurdles to clinical stationing. This study accentuates the requirement for a unified One Health strategy — coordinating veterinary, human, and environmental health policy — alongside evidence-based antibiotic stewardship, enhanced

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wastewater management, and molecular surveillance to curb the ongoing AMR crisis driven by HGT.

Keywords: antimicrobial resistance, antibiotic resistance genes, CRISPR-Cas9, conjugation, mobile genetic elements, one health, transformation, transduction

1.INTRODUCTION

The discovery and widespread clinical use of antibiotics initially generated optimism that bacterial infections could be effectively controlled and even eliminated. However, this optimism was soon proven to be misplaced. Only a few years after the clinical introduction of penicillin, bacterial resistance emerged in several hospital settings [1]. According to the global surveillance data published by the World Health Organization (WHO), antibiotic-resistant infections directly remain attributable to more than 1.2 million deaths in 2019, with the majority occurring in low- and middle-income countries (LMICs) — a figure that has continued to rise [2]. The first strains of penicillin-resistant *Staphylococcus aureus* were isolated in 1947. With the emergence of methicillin in 1961, only two years after its introduction, MRSA (methicillin-resistant *S. aureus*) also emerged. The rapid development of resistance after the introduction of antibiotics became a recurring issue, as it still is today [3]. Bacterial antibiotic resistance refers to the development of new mechanisms in bacteria to withstand previously effective antimicrobial agents [1]. Resistance mechanisms include the enzymatic degradation of antibiotics, target site modification, increased efflux pump expression, and decreased cell wall permeability [4]. It is mostly made possible by HGT, which enables the bacteria to obtain genetic material from other organisms without really becoming their progeny [5]. Unlike vertical gene transfer, which occurs through reproduction from parent to offspring, HGT enables bacteria to exchange genetic material across species boundaries, markedly accelerating the evolution and dissemination of antibiotic resistance [6].

HGT occurs primarily through three mechanisms—conjugation, transformation, and transduction—each with distinct characteristics. These progressions can result in bacterial strains that are resistant to several antimicrobial agents due to the instantaneous acquisition of multiple resistance genes. Resistance to antibiotic therapy becomes particularly annoying when the bacteria can gain and disseminate the genetic material

associated with resistance through HGT [7]. The economic impact is equally staggering and at WHO 2024, it was estimated that antibiotic resistance results in over $100 billion in lost economic productivity and healthcare expenditures, annually [8]. The socioeconomic environment, coupled with the healthcare system in Pakistan, places the country at serious risk in terms of antibiotic resistance. The country experiences a perfect storm of factors: easily accessible antibiotics without prescriptions, poor infection control policies in healthcare facilities, inadequate sanitation, limited diagnostic services, and high population density in urban centers. Research done in major cities in Pakistan shows exceedingly high prevalence rates of multidrug-resistant (MDR) bacteria in both community and hospital settings [9].

Over the past four decades, inadequate regulatory policies and the unregulated sale of antibiotics in Pakistan's retail pharmacy industry have exerted substantial selective pressure. With more than 80% of antibiotics supplied without a legal prescription, excessive self-medication, improper dosage, and early treatment termination are widespread. These variables aid in the development of resistant strains, leading to extended illnesses, amplified treatment failure, and a substantial financial burden on the healthcare system [10]. Bacterial population density, temperature, nutrient availability, pH, and the presence of antibiotics are the environmental factors exerting significant influence on the rate and efficiency of HGT. Their interaction is based on mobile genetic elements (MGEs), which distribute antibiotic resistance genes (ARGs) from one bacterial cell to another. Crucial genetic elements involved in HGT include plasmids, integrons, transposons, and genomic islands that serve as effective vehicles for acquiring, preserving, and distributing resistance genes. This structural organization allows for the aggregation of multiple resistance gene clusters, permitting bacteria to develop resistance against several classes of antibiotics simultaneously [5].

The way genes move horizontally depends greatly on the environment. Coming into contact with antibiotics, weak patients, and many invasive devices, as in intensive care units, often increases the chances of bacteria exchanging genes. Effluent discharge facilities can enhance the spread of resistance genes among bacteria by bringing together various bacterial species and offering nutrient-rich conditions that encourage their interaction and gene exchange [11]. Antibiotics used in animal farming facilitate HGT

by fostering the growth of resistant bacterial strains, which increases the likelihood of resistance genes spreading among microbial populations. Resistant bacteria and left over antibiotics in manure exert pressure on agricultural soil. As a result, a reservoir of ARG develops, which can be transferred to human pathogens via various transmission pathways, such as direct contact, food chains, water systems, or environmental exposure [12]. As a result of globalization, antibiotic-resistant bacteria and resistance genes now spread around the globe more rapidly. People, animals, and food traveling across nations allow infectious strains to be spread fast to several continents. For example, NDM-1-producing bacteria have been detected globally, largely due to their international dissemination, while colistin resistance genes such as *mcr-1* have also spread worldwide, facilitated by global trade and travel [13].

Unlike previous reviews that describe HGT mechanisms in isolation, this work critically compares conjugation, transformation, and transduction in terms of their relative clinical frequency, host range, and resistance gene payload capacity. It further evaluates environmental and anthropogenic amplifiers of HGT within an integrated One Health framework and provides a critical appraisal of emerging interventional strategies and their translational readiness.

2. METHODOLOGY

A thorough literature review was organized to evaluate the processes affecting HGT in depth as well as their role in antimicrobial resistance. The databases used were Google Scholar, ScienceDirect, and PubMed/MEDLINE. Boolean operators and the following MeSH-aligned terms: ("conjugation" OR "transduction" OR "transformation") AND ("antimicrobial resistance" OR "AMR") AND ("horizontal gene transfer" OR "HGT") AND ("wastewater" OR "environmental reservoirs" OR "plasmid" OR "biofilm") were employed as search strategy. The articles published from 2012 to 2026 were included, with priority given to comprehensive reviews, peer-reviewed original research articles, and global surveillance reports from ECDC, WHO, and national health agencies. A preliminary screening of titles and abstracts identified 312 potentially relevant articles. After removing duplicates (n = 47] and applying the following exclusion criteria — (i) non-English language publications without verified translations, (ii) studies lacking molecular validation of resistance mechanisms, and (iii) grey literature without peer review — a

final set of 88 references were included. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework guided the selection process. Since quantitative synthesis was not possible due to the heterogeneity of study designs and variables, no formal meta-analysis was carried out.

2. MECHANISM OF HORIZONTAL GENE TRANSFER

2.1. Conjugation-Mediated Resistance Transfer

In clinical practice, conjugation is thought to be a very effective and efficient HGT process. Bacteria exchange genetic material in this process through direct physical touch [14]. The process starts with tiny plasmids, which are bacterial chromosome extra-chromosomal DNA units that multiply on their own. These plasmids frequently carry genes that encode virulence factors, antibiotic resistance, and metabolic functions. Plasmids are transferred from donor to recipient cells through a tube-like structure called a pilus [15]. The gene products encoded by plasmid-borne *tra* (transfer) genes are responsible for assembling the pilus. This structure connects bacterial cells and facilitates the transfer of single-stranded plasmid DNA from the donor to the recipient. Upon entry into the recipient cell, the single-stranded DNA rapidly replicates into a double-stranded plasmid. This newly acquired plasmid often carries genes conferring resistance to antibiotics, such as β-lactams and fluoroquinolones [16].

Bacteria carry resistance genes and share them with other bacteria through the process of conjugation and they become resistant to certain antibiotics, the most common are lactams and fluoroquinolones [17]. Antibiotic resistance through gene transfer is a serious problem. The gene *blaNDM-1* encodes the New Delhi metallo-β-lactamase-1 (NDM-1], an enzyme capable of hydrolyzing carbapenems. Since *blaNDM-1* is often located on plasmids, it can be horizontally transferred between bacteria, facilitating the spread of carbapenem resistance [18]. In 2018, a study showed that resistance gene was found in *Klebsiella pneumoniae* in a hospital of Germany. After a few weeks, the transference of this gene to other bacteria including *Escherichia coli* and *Enterobacter cloacae* resulted in treatment failure and even deaths.

The *mcr-1* gene confers resistance to colistin by encoding a phosphoethanolamine transferase enzyme that alters the lipid, a component of lipopolysaccharides, hence reducing colistin binding affinity. This gene

is also transmitted through plasmids found in *E. coli* and isolated from food, people, and animals [19]. Surveillance in China showed that 15% of retail chicken meat contained *E. coli* carrying the *mcr-1* gene, featuring the zoonotic transmission hazard of resistance determinants from animals to humans [20]. Resistance also spreads through integrons. These are genetic elements that capture, integrate, and express gene cassettes, including ARGs, thereby enhancing bacterial adaptability under selective pressure. In the case of sulfonamides and trimethoprim, resistance is commonly mediated by *sul1* and *dfrA* genes, respectively [21]. A study conducted in Spain investigated wastewater treatment plants (WWTPs) and revealed the presence of bacteria harboring conjugative plasmids carrying integrons, which helped the procurement and spread of ARGs [22].

Human and environmental factors also cause antibiotic resistance through conjugation. To keep animals healthy, antibiotics such as tetracycline are given to them. The said antibiotic kills harmful bacteria but it also contains tetracycline resistance genes *tet*(M) or *tet*(O). Conjugative plasmids carrying these genes can be transferred to other bacteria through HGT, thereby facilitating the spread of antibiotic resistance [23]. In 2020, a study from U.S. pig farm showed that 80% of *E. coli* had plasmids with *tet(M)*. The resistance gene goes into animal waste and from the animal waste it transfers into the soil. In this way, bacteria in the environment also become resistant. Some bacteria also contain metal resistance genes such as *czc* for zinc and *pco* for copper. These metals create pressure when they are in the soil. Only those bacteria that contain a metal resistance gene can survive in the soil [24].

2.2. Transformation and Natural Competence

Transformation is the process by which bacteria acquire genes from the environment and incorporate them into their DNA. In transformation, there is no requirement for direct cell-to-cell contact. Instead, it relies on the release of DNA from lysed cells and the ability of recipient bacteria to absorb and integrate this extracellular genetic material [7]. This process is particularly common in naturally competent bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Streptococcus pneumonia* which have developed specialized equipment to control the uptake of DNA [25]. In naturally competent bacteria, competence-stimulating peptides (CSPs) act as signaling molecules that cause the production of DNA uptake machinery. For instance, *S. pneumoniae* internalizes extracellular DNA by

means of the ComABCDE quorum-sensing system [26]. Once ingested, this exogenous DNA undergoes a homologous recombination to integrate into the host genome, hence conferring survival benefits like antibiotic resistance. Since its discovery in clinical isolates in the 1970s, the *penA* gene—which modifies penicillin-binding proteins (PBPs) to decrease antibiotic affinity—has spread throughout the world through transformation [27].

Acinetobacter baumannii's natural metamorphosis makes it easier for the organism to absorb carbapenemase genes from its surroundings [28]. Researchers in Thailand found a new *penA*-60 gene in *N. gonorrhoeae* strain FC428 in 2018. This gene was probably acquired by interaction with other benign bacteria. Alterations in penicillin-binding proteins (PBPs), mediated by specific resistance genes, reduce the binding affinity of ceftriaxone, rendering the antibiotic ineffective [29]. *A. baumannii* is a notorious nosocomial pathogen characterized by its genomic plasticity and ability to persist on inanimate hospital surfaces, facilitating the spread of healthcare-associated infections (HAIs) [30]. Resistance in carbapenem-resistant *A. baumannii* (CRAB) is primarily mediated by the acquisition of carbapenemases, such as OXA-23-like (*bla*OXA-23] and *bla*NDM-1, through HGT events. Recent molecular surveillance has confirmed that surfaces in hospital environments, particularly in Intensive Care Units (ICUs), act as significant reservoirs for multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, which acquire resistance determinants by taking up extracellular DNA fragments present on contaminated medical equipment [30]. Bacterial genetic modification also depends upon environmental factors. Bacteria living in biofilms, as well as in soil and aquatic systems, have extracellular DNA which is comparatively resistant to being broken down by DNases and other nucleases, thus enduring for a very long time and promoting HGT [32]. Gene transfer is also facilitated by WWTPs; *E. coli* present in the sludge gets the blaCTX-M-15 ESBL gene from free bacteria. The medical significance of transformation can be seen in the cases of chronic infections. For instance, in patients suffering from cystic fibrosis, *P. aeruginosa* can undergo transformation and acquire resistance genes from visiting or resident bacteria or biofilm DNA [33].

A study done in 2019 reported that around 30% of *P. aeruginosa* drug-resistant strains isolated from cystic fibrosis patients' lungs acquired

resistance-associated mutations through natural transformation [34]. Due to continued antibiotic use, certain strains survive and multiply, leading to an increase in DNA in the mucus of the lungs. Efforts to inhibit bacterial transformation are facing significant challenges due to the complexity of natural competence mechanisms and the protective nature of environmental reservoirs [35]. Biofilms are inherently difficult to eradicate using conventional antibiotics, as they protect embedded bacteria and extracellular DNA from antimicrobial agents. However, recent strategies employing nanoparticles conjugated with engineered DNases demonstrated promising results in disrupting biofilm structure and degrading protective extracellular DNA [36]. DNase-loaded nanoparticles used in a 2022 study helped to remove extracellular DNA in *S. pneumonia* biofilms, cutting down transformation rates by 70% and desensitizing the bacteria to penicillin. In addition, phage-delivered CRISPR-Cas systems are being used to break down resistance genes in environmental DNA before bacteria can take those in [37]. Overall, transformation strongly supports the increase of antibiotic resistance, mainly in complicated infections and outdoor environments. This can be tackled by methods that keep DNA from staying inside the body, blocking signal pathways, or targeting the genetic materials floating in the environment [7].

2.3. Transduction via Bacteriophages

Transduction occurs when bacteriophages (viruses that infect bacteria) transfer genetic material from one bacterial cell to another during their replication cycle. Unlike conjugation and transformation, transduction does not require direct cell-to-cell contact or the uptake of free environmental DNA. Instead, DNA transfer from one bacterium to another is made possible by bacteriophages acting as vectors [38]. Transduction can be done in two ways: generalized transduction is the type in which any portion of the bacterial genome can be transferred, whereas specialized transduction is the type which involves the transfer of only specific genes situated near the prophage integration site [39]. This is the mechanism which significantly contributes to the distribution of ARGs among pathogenic bacteria, such as *S. aureus* and *P. aeruginosa* [40].

Bacteriophages utilize the host bacterium's cellular machinery to synthesize new viral components [38]. Occasionally, phage packaging machinery mistakenly incorporates the fragments of the host's DNA into the latest viral capsid, typically around 5–10%, instead of phage DNA.

When these 'transducing particles' attach to a host bacteria, they inject its DNA into the new cell. If the DNA joins the recipient's DNA at the same position through homologous recombination, the bacteria gets new genetic features. Phage 80α in *S. aureus* is involved in spreading the *mecA* gene (methicillin resistance), leading to serious cases of global MRSA (methicillin-resistant *S. aureus*). When lysogeny takes place, temperate phages place their DNA into genes located at a special place, known as *attB* sites, in the bacteria's genome. When a phage is reactivated (by stress), it carefully removes its DNA, occasionally including nearby host genes. Erythromycin resistance in *S. aureus* occurs because phage Φ11 moves the *ermB* gene nearby the *attB* site [41].

2.4. Mobile Genetic Elements: Vehicles of HGT

Mobile genetic elements (MGEs) constitute the molecular infrastructure upon which all three HGT mechanisms depend. Plasmids are extrachromosomal, self-replicating DNA molecules classified by incompatibility (Inc) groups, which determine their host range and replication compatibility [42]. Clinically, IncF plasmids are the most prevalent carriers of blaKPC in North America, while IncX3 plasmids predominantly carry blaNDM-1 in Asia, and IncI2 plasmids are the primary vehicles of mcr-1 globally [43]. Plasmids can carry DNA payloads exceeding 500 kb, enabling the co-transfer of resistance genes, virulence factors, and metal tolerance loci in a single conjugation event [44]. Integrons are genetic elements that capture and express mobile gene cassettes through a site-specific recombination system. Class 1 integrons are the most clinically significant, detected in 40-50% of clinical Gram-negative isolates globally. These commonly carry cassettes encoding resistance to aminoglycosides, trimethoprim, sulfonamides, and chloramphenicol. Class 2 integrons are more prevalent in agricultural and food-animal associated isolates. Crucially, integrons are typically embedded within transposons or plasmids, coupling their resistome with conjugative mobility [45]. Transposons mediate ARG mobility via two mechanisms: replicative transposition (copy-and-paste, Tn3 family) and cut-and-paste transposition (IS element-based composite transposons, such as Tn10 carrying tet(B) tetracycline resistance genes) [46]. Critically, composite transposons can mediate the integration of ARGs directly into the bacterial chromosome, making resistance non-eliminable by plasmid curing alone — a fundamental limitation for CRISPR-based therapeutic

strategies. Genomic islands (GIs), such as the resistance island AbaR in *A. baumannii*, represent large-scale MGEs [10–500 kb) which carry multiple resistance gene clusters simultaneously and can be acquired en bloc through HGT, thus enabling the rapid emergence of XDR phenotypes [47]

2.5. Comparative Analysis of HGT Mechanisms in Clinical AMR Dissemination

Among the three primary HGT pathways, conjugation is the dominant driver of clinical AMR spread, accounting for approximately 70% of plasmid-mediated resistance dissemination among clinical Enterobacteriaceae [48]. It is capable of transferring large DNA segments (up to 500 kb), operates across a broad inter-species and inter-genus host range, and persists under antibiotic selective pressure. Transformation plays a major role in ARG accumulation in environmental and biofilm contexts and is most clinically significant in naturally competent nosocomial infections, including *A. baumannii, S. pneumoniae,* and *N. gonorrhoeae* [49]. Despite its limited DNA payload capacity, transduction plays a disproportionate and mechanistically unique function in *S. aureus*, where phage 80α-mediated transduction contributes to the global spread of MRSA and mecA. The design of intervention strategies is directly influenced by these mechanistic differences, with DNase-mediated extracellular DNA (eDNA) degradation being most effective in inhibiting transformation in biofilm environments, with anti-conjugation strategies (including pilus inhibitors and plasmid incompatibility exploitation) being particularly relevant in clinical settings, and engineered phage therapy targeting transduction-mediated dissemination of antibiotic resistance in pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) [38]. Table 1 gives a summary of these 3 mechanisms across key parameters.

Table 1. Comparative Overview of Three Primary HGT Mechanisms in Clinical AMR Dissemination

Parameter	Conjugation	Transformation	Transduction	Clinical Relevance
Host range	Broad; inter-species, inter-genus	Limited to naturally competent species	Based on the range of phage receptor	The widest clinical host range is conjugation.

Parameter	Conjugation	Transformation	Transduction	Clinical Relevance
Key clinical pathogens	*E. coli, Enterobacter spp K. pneumoniae,*	*A. baumannii, S. pneumoniae, N. gonorrhoeae*	*S. aureus* (MRSA), *P. aeruginosa*	All are WHO priority pathogens
Key resistance genes	blaNDM-1, mcr-1, blaKPC, tet(M)	penA, blaOXA-23, blaNDM-1	mecA, ermB, blaVIM-2	Conjugation: broadest ARG repertoire
Primary anti-HGT intervention	Pilus inhibitors; CRISPR plasmid curing	DNase nanoparticles; competence inhibition	Engineered lytic phages; CRISPR-Cas delivery	All remain preclinical

The above described molecular machinery of conjugation, transformation, and transduction does not function independently. Environmental factors, such as bacterial cell density, biofilm architecture, sub-inhibitory antibiotic concentrations, and food availability have a significant impact on the frequency, efficiency, and clinical outcome of each route. Important environmental reservoirs and human activities that selectively enhance HGT occurrences and turn localized resistance development into a worldwide AMR epidemic are examined in the section that follows.

2.6. Environmental Reservoirs and Anthropogenic Drivers

HGT is a process which is significantly influenced both by human activities and environmental factors, rather than being a biological phenomenon solely. While genetic exchange is facilitated by the molecular processes of conjugation, transformation, and transduction, anthropogenic forces which produce habitats that favor resistant bacteria cause ARGs to spread quickly throughout ecosystems [40]. Natural ecosystems have become reservoirs of resistance determinants because to the overuse of antibiotics in clinical and agricultural contexts, as well as pollution from urban and industrial sources. For example, bacteria carrying ARGs on MGEs, such as plasmids and integrons, are enriched when sub-therapeutic antibiotic dosages are present in cattle feed [50]. Similarly, by stabilizing

plasmids that include both metal tolerance and ARGs, heavy metals like copper and zinc, which are frequently employed in manufacturing and agriculture, co-select for resistance [51]. Resistance genes acquired in one niche can quickly spread to others due to the interdependence of human, animal, and environmental microbiomes—a fundamental component of the One Health paradigm. For instance, WWTPs serve as 'gene supermarkets,' where microorganisms from homes, farms, and hospitals share genetic information [52]. This section examines how anthropogenic activities and environmental reservoirs work together to enhance HGT, contributing to the global multidrug resistance epidemic.

Figure 1. Global Distribution of Antibiotic Resistance Gene Dissemination.

The map illustrates the correlation between ARG prevalence and regional antibiotic use patterns. ARG prevalence data was derived from WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) and published metagenomic surveys. Scale indicates the percentage of clinical or environmental isolates testing positive for at least one critical ARG category.

The global spread of antibiotic resistance genes varies significantly across countries and closely correlates with antibiotic usage in each region (Figure 1). The prevalence of antibiotic resistance genes is alarmingly high

in countries such as India, with rates ranging up to 85% in large part due to the overuse of carbapenem antibiotics in clinical settings. Physicians in the Indian ICUs tend to prescribe carbapenems when they are not medically necessary [53]. The practice provides optimal environments in which bacteria such as *Klebsiella pneumoniae* can acquire the blaNDM-1 gene and consequently transfer this gene to other bacteria species [54]. China has a high resistance rate of 78% which can be attributed to the extensive use of colistin in animal feed. Prior to its banning by the government in 2020, studies showed that 15% of chicken meat sold in Chinese markets contained *E. coli* along with the mcr-1 gene, which confers colistin resistance. This fact highlights the possibility that using antibiotics in livestock can lead to problems associated with antimicrobial resistance [55].

The aforementioned environmental reservoirs act as evolutionary incubators and gene grocers, directly contributing to the clinical AMR epidemic. The resistance determinants that build up in hospital biofilms, agricultural soils, and WWTP sludge are not ecologically inert; they re-enter clinical settings through contaminated food chains, water supplies, and healthcare personnel, where they show up as the policy and therapeutic failures discussed in the next section.

2.7. Wastewater Treatment Plants (WWTPs)

WWTPs serve as key convergence points of ARGs, with inputs of hospital effluents, pharmaceutical waste, and domestic sewage. Although the treatment processes are advanced, in most cases, WWTPs are unable to completely degrade antibiotics or kill resistant bacteria, creating a nutrient-rich environment in which HGT thrives [56]. In a landmark study, identical blaCTX-M-15 genes (encoding extended-spectrum β-lactamases) were detected in *E. coli* isolates in hospital sewage, retail meat, and farm animals, implicating WWTPs as a focal point of cross-ecosystem gene flow [57]. Competence genes are frequently enhanced by high bacterial density and stress conditions (e.g., oxidative stress) which allows conjugative plasmids to carry out conjugation, including blaNDM-1 (carbapenem resistance) and mcr-1 (colistin resistance) as frequently encountered examples [58].

2.8. Agricultural Systems

The ARGs, extracellular DNA (eDNA), and the leftover antibiotics become saturated in agricultural soils that are amended with manure that has been sprayed with antibiotics. According to a study on U.S. swine farms

conducted in [2020], 80% of *E. coli* isolates harbored conjugative plasmids containing tet(M) such as *Pseudomonas putida* which transferred to soil bacteria via transformation [59]. Naturally competent bacteria (which can acquire resistance genes into their genome) can be enabled to do so by the persistence of eDNA in the soil stabilized by divalent cations (Ca 2 +, Mg 2 +) and organic matter. Indicatively, *Acinetobacter baumannii* acquired blaOXA-23 (carbapenemase gene) in *P. aeruginosa* eDNA in contaminated Brazilian ICU soils, causing untreatable outbreaks [60].

2.9. Aquatic Systems and Biofilms

Biofilm formation in riverine and marine ecosystems contaminated with agricultural runoff or urban waste results in HGT rates 10-fold higher than in planktonic cells [61]. *P. aeruginosa* biofilms in cystic fibrosis patients, for instance, accumulate eDNA carrying mutations in gyrA (fluoroquinolone resistance) and ampC (cephalosporin resistance), which are easily transferred between adjacent cells. Likewise, phage-mediated transduction of blaVIM-2 in Seoul hospital water systems was a contributor to the outbreaks of carbapenem-resistant *P. aeruginosa* in burn units [37].

2.10. Globalization and Human Activities

Travel and trade across countries enable the rapid global spread of antibiotic resistance bacteria and resistance genes and how local resistance issues become global concerns in short periods of time. Translocation of people, animals, and goods across geographical borders offers numerous avenues through which resistance genes can be transmitted to different geographical areas [62]. International transmission of hospital-acquired resistant bacteria can be facilitated by medical tourism, whereby patients travel to other countries in search of healthcare services. Patients who contract multidrug-resistant infections during medical treatments in foreign countries can be vectors of introducing new resistance determinants to their home countries. This has been reported to spread with the dissemination of New Delhi metallo-betalactamase (NDM-1], introducing bacteria of the Indian subcontinent to other world regions [63].

3. CLINICAL AND POLICY IMPLICATIONS

The environmental ARG reservoirs described above — functioning as evolutionary incubators and gene supermarkets — continuously fuel the clinical AMR crisis. Resistance determinants amplified in WWTP sludge, manure-amended soils, and hospital biofilms re-enter clinical settings

through contaminated water supplies, food chains, and healthcare worker transmission, materializing as the treatment failures and attributable mortalities documented in the following section.

3.1. Therapeutic Limitations in Resistance Management

Cross-species resistance results through the process of horizontal gene transfer (HGT), whereby antibiotic resistance genes are transferred from a limited number of resistant bacterial strains to previously susceptible bacteria of different species, resulting in the spread of resistance to antibiotics that were once effective against those susceptible organisms [64]. The ICU rates of septic shock in Pakistan have resulted in the doubling of the usual rates of mortality, according to [10]. According to the said research, families have suffered greatly and healthcare system is finding it difficult to cure infections that were previously treatable using conventional methods. These treatment difficulties are worsened by the absence of sufficient diagnostic instruments. According to the available data, more than 75% of Pakistani district hospitals are unable to quickly identify resistance genes using MALDI-TOF or PCR techniques [65]. Clinicians frequently treat multidrug-resistant illnesses with ineffective empirical medications due to the lack of diagnostic tools. When the mcr-1 gene is found, colistin must be rationed, which has led to an increase in the prevalence of suboptimal treatment plans. This has altered the landscape of treatment, with drug shortages now determining treatment choices, rather than patient health [66].

While free DNA released from lysed cells is taken up in transformation events, conjugative plasmids can only be transported between bacteria when they come into contact, as demonstrated by the mechanisms for resistance gene transfer within hospitals. In ICUs, where antibiotic use is high, bacteriophages transfer resistance traits from one species of bacteria to another, creating complicated resistance patterns.

3.2. Economic Burden Quantification

In addition to raising healthcare expenses, HGT-mediated resistance creates issues across a wide range of socioeconomic sectors. Global healthcare systems are severely impacted financially by antimicrobial resistance, which results in longer hospital stays, higher treatment costs, and decreased productivity. Resources that could be used to support vital public health services such as immunization campaigns and rural healthcare

facilities would otherwise be diverted to treat resistant illnesses [67].

Healthcare systems are severely impacted financially by antimicrobial resistance (AMR), especially in low- and middle-income countries like Pakistan. If left unchecked, AMR is predicted to result in a cumulative economic loss of up to $100 trillion worldwide by 2050 [68]. According to research, resistant bloodstream infections dramatically raise direct healthcare expenditures in Pakistan, with additional inpatient costs in tertiary care settings averaging US$34 per case [69]. The resources available for other crucial public health services, such as immunization programs, the growth of primary healthcare, and the treatment of chronic illnesses, are constrained by these rising costs. Therefore, improving AMR detection and stewardship is both clinically and financially necessary for long-term health systems.

3.3. Molecular Intervention Strategies

The aforementioned governance deficiencies and therapeutic failures highlight the critical need for radically new strategies that target not only resistant microorganisms but also the mechanisms by which resistance spreads. The next section assesses new molecular tactics that directly obstruct conjugation, transformation, and transduction. This is a fundamentally new intervention paradigm that tackles the underlying mechanism of resistance propagation, instead of its effects.

Although comparable efficacy has not yet been shown in animal models or human clinical trials, CRISPR Cas9 systems have demonstrated remarkable success in eliminating *blaKPC* 2 plasmids (exceeding 94% curing efficiency *in vitro*). These figures reflect *in vitro* conditions in clinical isolates of *E. coli* and *K. pneumoniae*, restoring carbapenem susceptibility in enterobacterial pathogens [70]. By decreasing plasmid uptake in bacterial cells, experimental systems show that some nanoparticles, particularly metal oxide nanoparticles like TiO_2 and CeO_2, can greatly limit the transformation of antibiotic resistance genes [71]. However, these observations are limited to *in vitro* or environmental conditions, while reliable data on DNase-functionalized nanoparticles in animal or human models remains unavailable.

Table 2. Summary of Anti-HGT Approaches under Preclinical and Clinical Evaluation

Intervention	Target Mechanism	Documented Efficacy	Development Stage	
CRISPR-Cas9 Plasmid Editing	Plasmid curing by targeting resistance genes (e.g., *bla*$_{KPC}$)	98% elimination of *bla*$_{KPC-2}$ plasmids in clinical *E. coli* and *K. pneumoniae* isolates *in vitro*; *bla*$_{KPC-3}$ plasmids also cleared ~95% in some strains [72].	Laboratory proof-of-concept; limited *in vivo* murine gut models so far	Off-target editing; escape mutant selection; <50% in vivo delivery efficiency in murine gut
CeO_2 (metal-oxide) nanoparticles	Inhibiting natural transformation (ARG uptake)	Reduction of transformation frequency in *E. coli* at low concentrations; efficacy depends on dosage (*in vitro*) [71].	Experimental model systems (no animal or clinical data)	No animal or human data; complex nanomedicine regulatory pathway
Engineered phages	Blockade of resistance gene transduction	Clinical case reports of engineered phages against specific pathogens (e.g. *M. abscessus*) with positive outcomes; no controlled trial data for *mecA* [73].	Compassionate use and early clinical cases; not yet formal Phase I/II trials	Narrow host range; phage resistance evolution; not scalable to population level
Pilus inhibitors (anti-conjugation)	Blocking plasmid conjugative transfer	Conceptual/preclinical models; no quantitative efficacy data validated *in vivo* [74]	Preclinical	No in vivo data; potential microbiome disruption; broad-spectrum toxicity risk

Table 2 shows the experimental methods intended to interrupt different mechanisms of HGT. CRISPR-Cas9 systems targeting resistance plasmids like *bla*$_{KPC}$ have attained up to 95% elimination efficiency *in vitro*, with early validation in animal models [72]. Engineered bacteriophages show effectiveness in blocking *mecA* gene transduction in wound infections; however, limited host specificity remains an issue [73]. Whereas, pilus inhibitors suppress conjugative transfer in preclinical settings by targeting plasmid transfer machinery [75].

3.4. Limitations and Translational Challenges

Despite their preclinical promise, all current anti-HGT strategies face substantial barriers to clinical implementation that must be honestly evaluated. For CRISPR-Cas9-based plasmid curing, the central challenge is

in vivo delivery. The transfer of CRISPR cassettes using phage vectors into the complex polymicrobial gut or lung microbiome yields efficiencies far below 50% in mouse models, despite the great *in vitro* efficiency of phage mid-based delivery. Moreover, bacteria can quickly produce escape mutants by gaining anti-CRISPR proteins or changing phage surface receptors, which could make CRISPR-based therapies useless within the treatment window. Prior to practical translation, it is still necessary to define off-target Cas9 cleavage of the bacterial chromosome as a safety risk. DNase-functionalized nanoparticles face a different set of translational barriers: all current efficacy data are derived from *in vitro* or *ex vivo* biofilm models, with no pharmacokinetic, pharmacodynamic, or toxicological data from animal studies. Whereas, the regulatory pathway for novel nanomedicines remains complex and lengthy. Engineered bacteriophages remain restricted to compassionate use for individual patients with untreatable infections — far from scalable clinical deployment — and phage resistance evolves rapidly, particularly in biofilm communities. Pilus inhibitors remain at the conceptual and preclinical stage, with no validated *in vivo* efficacy data. It is, therefore, essential that all of these interventions be characterized as promising research-stage tools, rather than imminent clinical solutions, requiring sustained investment in translational science and early-phase clinical trials before they can enter routine practice.

3.5. Policy Gap Analysis

Numerous weaknesses within existing regulations facilitate antimicrobial resistance to emerge and spread in various sectors. Access to antibiotics remains poorly regulated, as most can still be purchased without a prescription, despite the WHO's recommendation that these drugs should only be dispensed with a valid prescription. Today, zoonotic surveillance is lacking because integrated systems to follow changes in drug-resistant genes among both animals and people rarely exist [76].

Table 3. One Health Governance Deficits

Policy Domain	Pakistan's Status	WHO Benchmark	Urgency Index
Antibiotic Access	90–97 % OTC availability [10]	Prescription-only	Critical
Farm Antibiotics	Growth promoters Used [77]	Therapeutic-only	Critical

Policy Domain	Pakistan's Status	WHO Benchmark	Urgency Index
WWTP Standards	Very limited treatment; mostly untreated [788]	Tertiary treatment	Medium
Diagnostic Infrastructure [NEW]	<25% of district hospitals have PCR/MALDI-TOF capacity	Routine molecular diagnostics at secondary-care level	High
AMR Surveillance Integration [NEW]	Fragmented; no unified One Health AMR database	Integrated human-animal-environment surveillance system	Critical

In Table 3, shortcomings in governance are listed in several major policy areas. Pakistan has just 3-10% of antibiotics available only by prescription, while WHO aims for 100%, so it needs urgent attention [10]. Farmers still use antibiotics, relying instead on growth promoters rather than the WHO's therapeutic-only approach, which has been given critical urgency status [77]. Rather than implementing the tertiary treatment recommended by the WHO, the U.S. permits wastewater treatment plants to discharge as partially treated or untreated water—a practice categorized as medium urgency [78]. Although we have more knowledge about antibiotics in agriculture, the use of growth promoters still outweighs that of therapies. As a result of this close association, animals' gut bacteria quickly gain resistance to drugs that may infect humans through the food people eat. Most wastewater treatment standards are not high enough, so most of the facilities just discharge untreated wastewater into local bodies of water, resulting in areas where HGT easily takes place.

3.6. Integrated One Health Framework

Resistance management works best when there are guidelines that connect human health, animal health, and the environment. It acknowledges that antimicrobial resistance brings together different disciplines and regions, so all stakeholders need to cooperate.

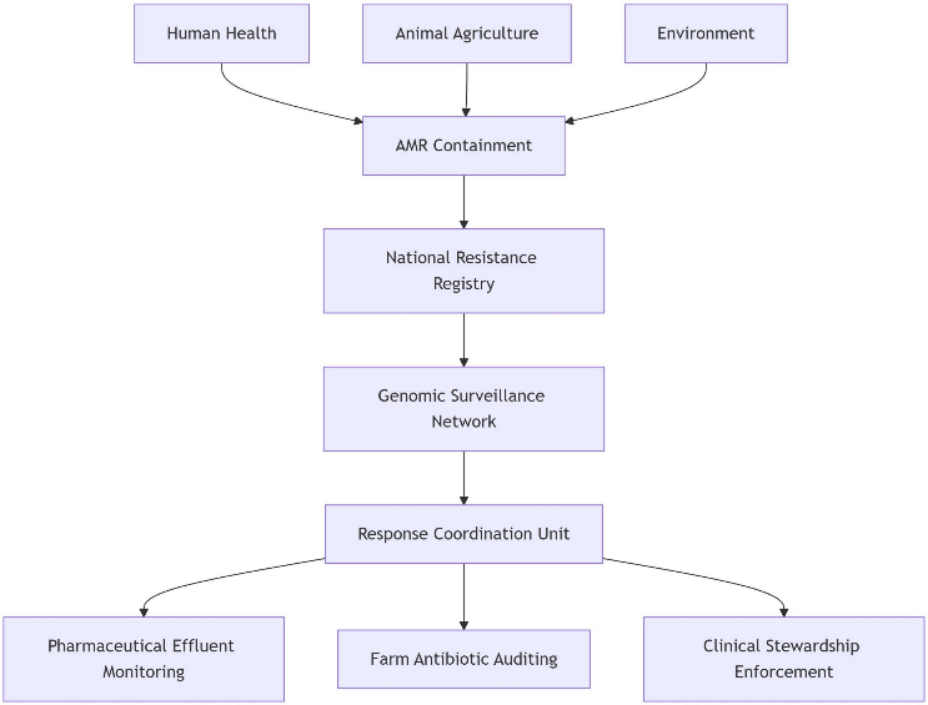

Figure 2. Cross-Sectoral One Health Implementation Model

Diagram illustrating resistance gene flow pathways between the human health (clinical), veterinary/agricultural, and environmental sectors. Arrows indicate unidirectional ARG transfer routes. Blue boxes represent sector-specific intervention points. The three inter-sectoral feedback loops — [1] clinical effluents to WWTP to community water, [2] agricultural antibiotic use to food chain to human carriage, and [3] environmental eDNA to nosocomial pathogen transformation — are explicitly annotated.

Coordination techniques involving shared surveillance systems, group response tactics, and uniform legal frameworks are shown in the above diagram. AMR must be actively controlled by coordinated policy frameworks and integrated data systems. Initiatives have been established with the goal of enhancing cross-sectoral communication by establishing data flow paths connecting Pakistan's burgeoning environmental, animal, and human health sectors. These frameworks facilitate the timely interchange of AMR-related data and aid in the harmonization of regulations [79]. By creating data flow channels between Pakistan's rapidly

expanding environmental, animal, and human health sectors, initiatives are being developed to improve cross-sectoral communication. These frameworks help to harmonize rules and enable the prompt exchange of AMR-related data [80]. To monitor the spread of resistance determinants including blaNDM and mcr genes, sentinel surveillance stations have been set up at wastewater treatment facilities in Karachi and across chicken farms in Punjab [81]. However, enforcement shortcomings and conflicting regulatory requirements continue to be major issues. To stop resistant bacteria from taking advantage of weaknesses in governance institutions, the Environmental Protection Agency (EPA) and the Drug Regulatory Authority of Pakistan (DRAP) must work together.

3.7. Implementation Roadmap

3.7.1. Transmission Dynamics. We can find numerous ways that resistance spreads throughout Pakistan by researching the transmission of resistant genes. Hospitals frequently employ antibiotics, which accelerates the evolution of germs and facilitates the transmission of illnesses among patients. Working with growth promoters in agricultural cattle causes resistance, which enables these germs to proliferate in food supply through afflicted animals.

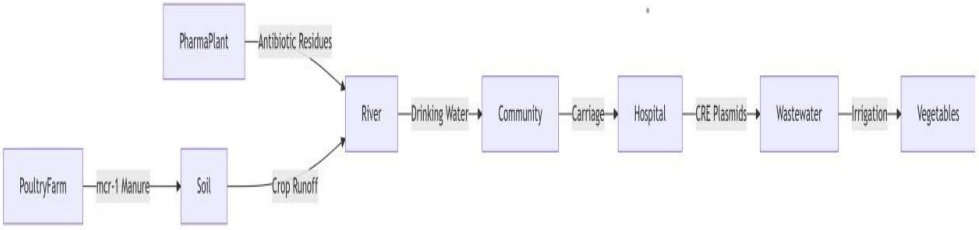

Figure 3. Resistance Gene Flow in Pakistan

This Flow chart shows the passage of ARGs between wastewater treatment facilities, hospitals, farms, and the community. Hospital discharge — of both patients and effluents — is the primary vector of clinical AMR to community settings. Agricultural transmission operates through the food supply and occupational exposure pathways. Inadequately treated wastewater creates environmental hotspots where all three HGT mechanisms operate simultaneously, amplifying the resistance gene pool available to community and clinical bacterial populations.

Figure 4 indicates how resistance genes move between hospitals, farms,

wastewater facilities, and the community in Pakistan. It demonstrates that people who are discharged from hospitals and healthcare workers can spread community-level resistance through such tools as discharge. Resistance from foods and occupational contact can demonstrate how antibiotic-resistant bacteria move from animals to humans via agriculture. By not properly treating wastewater, areas with high levels of antibiotic-resistant bacteria that share genetic information with a range of similar bacteria are created.

3.7.2. Tiered Diagnostic Strategy. The way diagnosis is carried out needs to account for large differences in resources across health services in Pakistan, while still making it possible to observe a broad range of cases. With this system, it is recognized that running advanced diagnostics everywhere would be too costly but important surveillance procedures can still be carried out.

Table 4. Resource-Adapted Tiered AMR Surveillance Framework for Pakistan

Facility Tier	Recommended Tools	Resistance Targets	Surveillance Output
Tertiary Hospitals	WGS, MALDI-TOF	NDM, KPC carbapenemases	National ARG genotype mapping; outbreak investigation
District Hospitals	Multiplex PCR, VITEK	ESBLs, colistin resistance	Regional resistance trends; empirical therapy guidance
Primary Clinics	CRP strips, UTI dipsticks [82]	Empirical therapy guidance	Community-level AMR burden estimation; stewardship data

Table 4 summarizes the approach to surveillance used in each type of facility. Tertiary hospitals need to add whole-genome sequencing and MALDI-TOF systems for *NDM* and *KPC* carbapenemases to their most important approaches [83]. Multiplex PCR and VITEK should be used in district hospitals to detect ESBLs and colistin resistance [84]. Data from

CRP strips and UTI dipsticks at primary clinics would support treatment choice and help analyze resistance in the country.

3.7.4. Sustainable Intervention Models. Financial restrictions and infrastructure limitations must be considered in risk-reduction efforts, while also supporting better resistance control via strategic market-based pricing. Incentivizing antimicrobial stewardship in agriculture through market-based premiums for antibiotic-free products may encourage better farming practices and reduce selective pressures. Pharmacists may link bundles of tests and medications for antibiotics, so that an infection is confirmed before antibiotics are administered [85]. Updating wastewater treatment with membrane bioreactor technology at 20 critical sites by 2030 will solve environmental problems at key outlets [86]. The plan focuses on assisting facilities with the greatest environmental impact in testing and demonstrating the effectiveness of advanced waste treatment [87].

3.8. Key Implementation Barriers

Dealing with growing resistance requires new ideas and enduring support from those who make decisions. The biggest immediate problem is how to pay for genome sequencing, as the typical laboratory budget cannot comfortably cover the sample fee [88]. It is almost impossible for regulators to ensure proper checks on pharmacies nationwide in Pakistan. Such institutional barriers can only be overcome when political leaders make strong commitments and formally coordinate their actions outside their usual ministerial domains. To overcome the challenge of group efforts, having a special authority leading cross-sector operations may be necessary.

3.9. Evidence-Based Projections

The evidence available suggests that, despite obstacles, a broad approach to resistance management can lead to major positive outcomes. Future clinically implemented CRISPR-based plasmid curing plans, if successfully scaled and translated from preclinical models, have the possibility to significantly reduce carbapenem resistance and improve long-term healthcare costs. The introduction of scale diagnostic approaches using UTI and CRP testing together can result in lesser unnecessary prescriptions of antibiotics and improve patient outcomes [82].

4. CONCLUSION

HGT leads to uncontrolled spread of antibiotic resistance via

synergizing conjugation, transformation, and transduction mechanisms across taxonomic and ecological boundaries. Genes associated with resistance development are often transferred via environmental reservoirs, such as wastewater treatment plants (WWTPs), farms, and biofilms. This is because of extensive use of antibiotics clinically, administration of growth promoters in livestock, and poor wastewater management. Pakistan works as a critical case study of the dangers posed by antibiotics' unregulated access, deficient diagnostic infrastructure, and selective agricultural pressures. While there are some newly discovered ways of dealing with it, such as CRISPR-based gene editing to remove plasmids and DNase nanoparticles to prevent HGT, success can only be achieved by applying these practices on a global scale. This problem can be dealt by adopting a holistic One Health approach where regulations in all medical, veterinary, and environmental fields must be coordinated. Using suitable testing methods and upgrading WWTPs are the prior steps involved in increasing antibiotics control and limiting their overuse. The number of drug-resistant diseases would grow if early actions are not taken worldwide, leaving current treatment methods useless. Hence, worldwide action supported by research is required.

Author Contribution

Ayesha Rasheed: writing-review & editing. **Komal Raja:** writing - original draft. **Nimra Shoukat:** writing - original draft. **Sidra Kaleem:** supervision, writing-review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

All data supporting the conclusions are derived from the published literature cited herein. No new primary datasets were generated or analyzed during this study.

Funding Details

No funding has been received for this research.

Generative AI Disclosure Statement

The authors did not use any type of generative artificial intelligence software for this research.

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