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ESBL-Producing *Escherichia coli*: The Role of *Plasmids* in Antibiotic Resistance

Aqsa Batool[a], Ali Raza[b], Samia Akmal[c], Sami Ullah[d], Mahad Ali Arshad[e], Ahmad Mateen[f], Sarfraz Ahmad[g], Mahnoor Shahid[h] Khansa Ahmed[i] and Sareen Fatima[j]

[a] Quality Assurance Officer, Department of Pathology, Shaukat Khanam Memorial Cancer Hospital and Research Center Lahore, Punjab, Pakistan
[b] Lab Manager, Department of Pathology Khawaja Muhammad Safdar Medical College, Sialkot, Punjab Pakistan
[c] Department of Biotechnology, Fatima Jinnah women University Rawalpindi, Punjab, Pakistan
[d] Department of Medical Laboratory Technology, Faculty of Allied Health and Biological Sciences, Ibadat International University, Islamabad Pakistan
[e] Department of Pathobiology, University of Veterinary and Animal Sciences, Lahore, Sub-Campus Jhang, Jhang, Pakistan
[f] Department of Medical Laboratory Technology (MLT), Riphah International University, Pakistan
[g] Health & Population Department, University of Health Sciences, Lahore, Punjab, Pakistan
[h] Department of Biosciences, Comsats University Islamabad, Pakistan
[i] Department of Zoology, GC Women University Sialkot, Punjab, Pakistan
[j] Allied Health Sciences, Bolan University of Medical and Health Sciences, Brewery Road, Quetta, Baluchistan, Pakistan

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Abstract

Background: Plasmids are the major route of the dissemination of resistance genes between species, sectors and geographical locations, and extended-spectrum β-lactamase (ESBL)-producing Escherichia coli are a critical health threat globally. **Objective:** Based on recent knowledge, this review intends to bring together the molecular epidemiology, plasmid biology, horizontal gene transfer mechanisms and therapeutic approaches in ESBL-producing *E. coli*, with a focus on the transmission dynamics in the realm of One Health. **Methods:** We searched the literature in PubMed, Scopus, and Web of Science from 2015 to 2026 using the keywords, "ESBL," "*E. coli*," "plasmids," "antibiotic resistance," "horizontal gene transfer," and "One Health". Articles were selected based on their relevance to understanding the nature of plasmid mediated resistance, epidemiology, and strategies for interventions. **Results:** ESBL-encoding plasmids vary in different species and contain a range of resistance determinants, such as blaCTX-M, blaTEM, blaSHV genes, which are frequently found together with aminoglycoside, quinolone and colistin resistance genes, with prevalence of IncF, IncI, IncN or IncHI2 plasmid types. The pandemic ST131 clone shows good plasmid-host adaptation that has different dominant subclades in different regions. Plasmids are very flexible in terms of the presence of mobile genetic elements (ISEcp1, IS26) that allow the capture and dissemination of resistance genes. Multidrug resistance and virulence determinants converged on epidemic plasmids and resulted in hypervirulent multidrug resistant strains. **Conclusions:** A systems biology approach to better understand plasmid biology, together with new technologies such as long-read sequencing and artificial intelligence, and the need to support the One Health approach, are all crucial to reducing the threat of ESBL-producing *E. coli*. There are potential treatment options that are directed towards resistance plasmids, but they need to be developed and verified.

Introduction

Multidrug-resistant bacteria make an important contribution to morbidity and mortality worldwide and antimicrobial resistance (AMR) is one of the public health challenges of the twenty first century. Of these pathogens, the most common and clinically important are third generation cephalosporin resistant enterobacterales, especially those harboring extended spectrum β lactamases (ESBLs). *E. coli* has been identified as a key source for this resistance and is a primary cause of community and healthcare associated infections as well as being a sentinel organism for AMR surveillance in human, animal and environmental sectors [1].

In 2015 the human carriage rate of ESBL-producing Enterobacterales in the world was estimated to be 14%, increasing by 5.4% each year over the last 20 years. This has spread into the animal sector, too, which illustrates the linkage in dissemination of resistance. ESBL-producing *E. coli* is classified as critical priority pathogen by the World Health Organization and novel approaches for monitoring and

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controlling its spread are needed. Mortality associated with infections due to these organisms also increases, due to the lack of appropriate therapy, resistance, and the paucity of new antimicrobials in development. ESBL evolution has been a consequence of adaptive response of bacterial populations to a selective pressure of the use of β-lactam antibiotics [2]. The early ESBLs (1980s) were mutations of penicillinases such as TEM-1 and SHV-1, and these were mostly found in hospitals. These early ESBLs shared with their progenitors the core structure with the addition of the ability to hydrolyse extended-spectrum cephalosporins, including cefotaxime and ceftazidime [3].

ESBL epidemiology has changed significantly since the advent of and worldwide spread of CTX-M enzymes. The ESBLs of the CTX-M type are different from TEM and SHV types and were originally found in environmental bacteria of the genus Kluyvera, having been mobilized onto *Plasmids* via the action of insertion sequence elements, in particular ISEcp1. There are currently more than 100 variants in the CTX-M family among which the most widespread in the world are CTX-M-15 and CTX-M-27. CTX-M enzymes have a strong preference towards the hydrolysis of cefotaxime and ceftriaxone over that of ceftazidime in contrast to the TEM and SHV variants [4]. The shift from TEM and SHV to CTX-M as the predominant ESBL type in community settings represents a key point in evolution of resistance epidemiology as the genes of CTX-M are much more mobile and successfully associated with *Plasmids*. Although less common worldwide, OXA-type ESBLs are emerging as a focus due to their link to carbapenem resistance, especially Acinetobacter baumannii and some lineages of Enterobacterales. In particular, the OXA-48 family has become a major concern because of its ability to hydrolyse a new class of antibiotics (carbapenems), and dissemination by plasmid. *Plasmids* have become the main disseminators of ESBL as they have a combination of properties that make them very successful as vehicles for resistance [5], [6]. They are an extrachromosomal DNA molecule that has its own self-replication mechanism and is maintained separately from the bacterial chromosome. These include transfer mechanisms which help them to spread both vertically to daughter cells and horizontally (conjugation) to non-related bacteria, turning isolated resistance cases into epidemic waves [7].

The success of ESBL-encoding *Plasmids* is because they can carry an array of resistance determinants and co-localize them generating multidrug resistance (MDR) phenotype after a single transfer. Such a genetic structure is advantageous in terms of selection because they will encourage the maintenance of the whole resistance complement if they are selected for any one trait. In addition, systems to control plasmid addiction, including toxin-antitoxin systems, provide plasmid stability by killing plasmid-free cells post-segregational and promoting persistence without selective pressure [8].

The plasmid-encoded transfer of CTX-M genes is a good illustration of these principles. blaCTX-M genes are often found associated with highly mobile insertion sequences, such as ISEcp1 and IS26, which enable the genes to be captured and mobilized to various different plasmid backbones. The *Plasmids* which have been formed, are IncF, IncI, IncN and IncHI2 etc., and these are found in global distribution - crossing over the geographic and the species barriers with ease [7].

Although there have been a few comprehensive reviews, there is a lack of knowledge on the integrated understanding of plasmid biology, host adaptation, One Health transmission dynamics, emerging therapeutic interventions, and specifically for ESBL-producing *E. coli*. Most of the reviews in the past have concentrated on either clinical epidemiology or molecular mechanisms alone, without dissecting the various factors involved in plasmid evolution, fitness of host, environmental reservoirs, regional transmission pattern etc.

This review is stimulated by several recent developments that include the rise of hybrid, multidrug resistant clones with high virulence, the co-location of resistance and virulence determinants on epidemic *Plasmids*, developments in the application of artificial intelligence and long-read sequencing technologies that have revolutionized the understanding of plasmid epidemiology and the urgent need for intervention strategies informed by a One Health approach. This manuscript, in contrast to current reviews, will be a systems-level synthesis that combines molecular epidemiology, plasmid biology, ecology, and applications to translation of therapeutic development especially for the 2022-2026 timeframe.

Methodology

- **Review Type**

The manuscript is a narrative review with the aim of summarizing the available information related to plasmidmediated antibiotic resistance in ESBL-producing *E. coli*. The narrative review approach was chosen to provide integrated synthesis of this multifaceted and interconnected subject area which would be best served by the narrative method as opposed to the more narrowly focused systematic approach.

- **Literature Search Strategy**

A thorough literature search was carried out for peer-reviewed articles published between January 2015 and June 2026 to identify relevant articles. These electronic databases were searched: PubMed/MEDLINE, Scopus and Web of Science Core Collection.

The search terms used were: ("ESBL" OR "extended-spectrum beta-lactamase" OR "SHV" OR "TEM" OR "CTX-M") AND ("*Escherichia coli*" OR "*E. coli*") AND ("antibiotic resistance" OR "antimicrobial resistance") and *Plasmids* OR plasmid mediated.

Further literature searches were performed on particular subjects: plasmid incompatibility groups, horizontal gene transfer, One Health transmission, ST131 epidemiology and therapeutic interventions (CRISPR-Cas and phage therapy, anti-conjugation molecules).

❖ Inclusion and Exclusion Criteria

Articles were included if they: (i) were published in peer-reviewed English language journals; (ii) discussed ESBL-producing *E. coli* or plasmid-mediated resistance in Enterobacteriaceae; (iii) presented original data or extensive reviews or meta-analyses applicable to the biology, epidemiology, transmission or therapeutic intervention against *Plasmids*; and (iv) were published between January 2015 and June 2026 (excluding seminal older references, where historically significant).

Articles were discarded if they: (i) did not specify *E. coli* species and/or were not related to the research question; (ii) discussed only resistance mechanisms on the chromosome and not the plasmid; or (iii) were not a research article (conference abstracts, editorials and opinion pieces without extensive data).

❖ Study Selection

Titles and abstracts were screened for relevance. Full texts of potentially eligible articles were assessed. Reference lists of included articles were manually searched to identify additional relevant publications (snowballing). A total of 358 articles were included in the final synthesis.

❖ Ethical Considerations

This review did not involve human or animal subjects; therefore, ethical approval was not required.

ESBL-Producing *E. coli*: Molecular Epidemiology

❖ Major Global Clones

Only a few successful clones of ESBL-producing *E. coli* have been able to spread globally, dominating the epidemiology of these organisms. The pandemic ST131 is the leading clone and has been considered as the most important associated with the worldwide rise in ESBL producing *E. coli* infections. The ST131 is thought to have three primary clades: clade A, clade B, and clade C, and clade C has additional subclades (C1-nM27, C1-M27, and C2). The C2 subclade is most common in North America and Europe and the C1-M27 subclade is most common in Japan and other Asian countries and is usually blaCTX-M-27[9].

The high evolutionary success of the ST131 clade C has been attributed to the ability to acquire the ESBL genes easily, the high virulence potential, and the ability of the ST131 Clade C to have a minimal fitness cost. Despite the core genome being conserved, accessory genome analyses have shown that the accessory genome of ST131 clades is highly dynamic and has a large amount of plasmid and mobile genetic element (MGE) activity. This adaptability enables fast response to alterations in selective pressures such as the gaining of new resistance determinants and the emergence of genomic areas specific to subclades [10].

Other clinically and environmentally important globally disseminated clones include ST10, ST410, ST38, ST648 and ST69. Among these, ST10 has been recognized as a One Health clone of concern, with strains found in both humans and animals as well as in the environment, and frequently containing CTX-M-55 ESBL genes, in various geographical settings [11].

❖ Regional Differences

Extensive regional variability has been observed in the prevalence of ESBL-producing *E. coli* clones and associated *Plasmids*, which likely varies due to differences in antibiotic consumption, healthcare services, environment and surveillance systems.

In Europe and North America, the ESBL producing *E. coli* has generally circulated between sectors and the transmission cycle within human, animal and environmental sectors is different. Human-to-human transmission of the ST131 subclade C2 is the major mechanism for clinical infections, and the ST131 C2 subclade is dominant in these areas. There is little evidence of cross-sectoral transmission in high-income countries with the livestock-associated lineages (e.g., ST10) rarely found in clinical populations and the clinical populations of poultry-associated clones not frequently found in livestock [11].

The situation is more complicated in Asia, where the development of dominant subclades can be seen in the region. ST131 has become predominant in Japan as the C1-M27 subclade which is characterized by the presence of subclade-specific genomic islands, which may be important in its persistence and dissemination. No matter in how many countries of Asia, studies have shown frequent intersectoral transmission with significant overlap between the number of humans, animal, and environmental isolates such as in India, Bangladesh, Thailand, and Vietnam as low and middle-income countries [12].

Africa is a hotspot with ESBL-producing *E. coli* extensively transmitted between ecological compartments as revealed by whole genome sequencing. Eastern Africa; genomic clusters resulted from the isolates from humans, animals and the environment, suggest recent transmissions and co-circulation within and between sectors, which contradicts the view of more compartmentalized circulation seen in high-income countries [13].

Increased problems with ESBL-producing *E. coli* in Pakistan and other South Asian countries are due to high carriage rates in the community, high antibiotic usage and poor infection control infrastructure. Reservoirs (sewage, agriculture) and epidemic *Plasmids* and international clones create a favorable situation for rapid spread of resistance [14].

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Plasmids Biology: More Than Mobile DNA

❖ Structure of Resistance *Plasmids*

Resistance *Plasmids* are complex genetic units that have evolved to be persistent, to replicate, and to spread. Minimal plasmid backbone consists of a replication origin (ori), where DNA synthesis begins and controls the copy number of *Plasmids*. Replication control systems, including antisense RNAs and iteron-based regulation, promote copy number homeostasis, balance of high copy number and metabolic cost of excessive plasmid replication [15].

Partition genes (par) facilitate correct segregation of the plasmid during cell division, whereby plasmid free daughter cells are not formed. Such systems are based on active partition mechanisms involving ATPases, like ParA and ParB, that target plasmid DNA to opposite cell poles before cell division. Plasmid stability is presumably enhanced by the presence of addiction systems, such as toxin-antitoxin modules (e.g., ccdAB, relBE) and restriction-modification systems, which kill post-segregant cells [16].

Transfer genes (tra): Conjugation machinery, type IV secretory system, for DNA transfer. Usually such genes are located on large parts of the plasmid DNA and the expression of such genes is highly regulated; i.e. conjugation takes place only under the right conditions [8].

❖ Major *Plasmids* Incompatibility Groups

Plasmids are classified into incompatibility (Inc) groups based on their inability to coexist stably within the same bacterial cell, a property determined by shared replication control mechanisms. Table 1 summarizes the major Inc groups associated with ESBL dissemination [4], [5]

Table 1: Representative *Plasmids* Incompatibility Groups Associated with ESBL Dissemination

Inc Group	Key Features	Common Resistance Genes	One Health Distribution
IncF	Low copy number, stable maintenance, toxin-antitoxin systems	*bla*CTX-M, *bla*TEM, *bla*SHV, *qnr*	Humans, animals, environment
IncI	High transfer efficiency, broad host range	*bla*CTX-M, *bla*TEM	Humans, animals, environment
IncN	Broad host range, mosaic structure	*bla*CTX-M, *bla*KPC, *bla*NDM	Humans, animals, environment
IncHI2	Large size, complex regulation	*bla*CTX-M, *bla*SHV	Humans, animals, environment
IncA/C	Broad host range, large cargo capacity	*bla*NDM, *bla*CMY, *rmtC*	Humans, environment
IncX	Small size, high copy number	*bla*CTX-M, *qnr*	Humans, animals
IncL/M	Conjugative, broad host range	*bla*OXA-48	Humans, environment

❖ *Plasmids* Evolution

By several mechanisms, *Plasmids* evolve and thus give rise to diversity in their genetic architectures amongst clinical isolates. Combining two or more *Plasmids* through cointegration events can result in a large composite replicon that contains a large resistance gene array. Recombination, both homologous and illegitimate, can lead to the reshuffling of genetic modules, which results in new combinations of res genes with backbone genes. Mosaic *Plasmids*: These capture segments of DNA from several sources which create chimeric *Plasmids* with new and increased functions. *Plasmids* with multiple origins of replication from different Inc groups offer increased stability and host range and thus they have a high dissemination potential [17].

Molecular Architecture of ESBL-Carrying *Plasmids*

❖ Resistance Islands

A genomic region of *plasmids* that may comprise a cluster of resistance genes which can be coordinately expressed and mobilized are called resistance islands. The genetic structure of these islands is typical, resistance genes being surrounded by IS elements, transposons and/or integrons involved in the acquisition and movement of the islands. Selection for any one resistance gene will promote the maintenance of the whole cluster since the multiple resistance genes will be retained in one single island, making MDR phenotypes [17].

❖ Mobile Genetic Elements

The engines behind plasmid evolution are mobile genetic elements (MGEs) which are able to capture, mobilize and disseminate resistance genes. The mobilization of ESBL genes has been a focus around small DNA elements, called insertion sequences (IS), such as IS26 and ISEcp1, which have the ability to move in the genome. Especially ISEcp1 which is often upstream of blaCTX-M and provides a promoter sequence that allows its expression [18].

Larger, MGEs called transposons, can move by cut and paste or replicative mechanisms and transport resistance genes as passengers. Integrons are a type of genetic element capable of acquiring various resistance determinants, by trapping gene cassettes through site-specific recombination. The integration of gene cassettes which carry the genes for resistance to antibiotics and other foreign drugs (like aac aminoglycoside acetyl transferases) into integrons and their transcription from a single promoter [19].

❖ *Plasmids* Plasticity

The ability of plasmid genomes to be flexible is the key to their success as resistance vehicles. Changes in patterns of gene expression as a result of structural rearrangements such as deletions, insertions, and inversions can generate new patterns of genes. The duplications of resistance genes can

increase the expression level, which will increase resistance phenotypes. Tandem duplications result in gene amplification which can account for a step-wise increase in resistance. The rearrangement of the genome, via homologous recombination between IS elements or other repetitive elements, can create diversity in plasmid structure and can free resistance genes from regulatory control [20].

Horizontal Gene Transfer: The Hidden Epidemic

- **Conjugation**

The most common way of horizontal gene transfer (HGT) mediated by *Plasmids* is conjugation, where plasmid DNA is directly transferred between cells via a type IV secretion system. A single strand of plasmid DNA is transferred between donor and recipient cells via a conjugation machinery, which is encoded by the tra genes. The transferred strand is replicated in the recipient to give a complete copy of the plasmid.

The frequency of conjugation depends on several factors, such as the availability of compatible recipients, expression of conjugation genes and the environment. The high level of conjugation found in laboratory strains can overestimate the frequencies of transfer in nature, especially in nutrient rich media. Conjugation frequencies tend to be lower in wild strains, which seems to be a tradeoff between plasmid transmission and fitness of the host. However, mathematical modeling has been able to characterize the dynamics of conjugation and create frameworks for understanding the interaction between the donor and the recipient population, plasmid replication and the environment [21].

- **Transformation**

Until now, the dissemination of plasmid-mediated resistance through uptake of naked DNA from the environment – known as transformation – has been regarded as less significant than plasmid dissemination by the conjugation process. But, there is growing evidence for transformation in some environments such as in biofilms and in the environmental niche where extracellular DNA is plentiful [22].

- **Transduction**

Another way for the transfer of a plasmid is by transduction, which involves bacteriophage packaging DNA and carrying them to new hosts. Transduction has received less attention than conjugation, but could possibly be involved in the spread of resistance, including between species.

- **Outer Membrane Vesicles**

The outer envelope vesicles (OMVs) are now known to be a means of horizontal gene transfer, a process by which plasmid DNA is transferred from a donor to a recipient cell. A new pathway for spread that involves OMVs protecting DNA cargo from the environment and transferring resistance genes to the various recipients [23].

- **Biofilm-Mediated Gene Transfer**

Biofilms are hotspots for horizontal gene transfer due to the high cell density, proximity of cells and sharing of signals, which are conducive to conjugation. Conjugation rates are higher in bacteria associated with biofilms than in planktonic bacteria, and biofilms act as a reservoir for resistance *Plasmids*, including their persistence in the environment [24].

The *Plasmids* Resistome

The plasmid resistome of ESBL-producing *E. coli* is rich and dynamic and includes a variety of determinants that aggregate to provide resistance to different classes of antibiotics. In this section an overview is provided of the diversity, the organization and the co-localization of resistance genes on *Plasmids*, with particular emphasis on the clinical importance of multidrug resistance (MDR) phenotypes [7].

- **ESBL Genes**

The ESBL genes are almost as diverse as the organisms that carry them; most commonly those genes are of the blaCTX-M, blaTEM, and blaSHV families, and are found in both human, animal, and environmental reservoirs. blaCTX-M-15 is the most prevalent ESBL determinant in all the reservoirs mentioned above. The genetic background of these genes is closely associated with mobile genetic elements, and that ISEcp1 is often located upstream of the blaCTX-M genes, while orf477 is located downstream, which helps in the capture and mobility of these genes. Other ISs (IS903, IS3, IS1380 families and IS26) have also been implicated in the acquisition/tracking of antibiotic resistance genes [18], [25]

ESBL dissemination genes (blaCTX-M, blaTEM and blaSHV) have been found on several plasmid incompatibility groups across One Health compartments including IncFIA, IncI1, IncY, IncFIB, IncN, IncFIC, IncX4, IncB/O/K/Z, IncHI1/2 and IncA/C. This variety of the plasmid backbones, which ESBL genes have been found on, highlights the versatility of these resistance genes and their ability to utilize multiple dissemination routes [4], [5]

- **Co-existing Resistance Genes**

The clinical significance of ESBL-encoding *Plasmids* is amplified by the frequent co-localization of resistance genes from multiple antibiotic classes. Table 2 presents the major resistance gene families co-localized with ESBL determinants on *Plasmids* [26], [27]

Table 2: Co-localized Resistance Genes on ESBL-Encoding *Plasmids*

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Gene Family	Representative Genes	Resistance Phenotype	Clinical Significance
Carbapenemases	*bla*NDM, *bla*KPC, *bla*OXA-48	Carbapenem resistance	Last-resort antibiotic resistance
Aminoglycoside	*aac*, *aph*, *ant*	Aminoglycoside resistance	Limits gentamicin, amikacin utility
Quinolone	*qnr*, *aac(6')-Ib-cr*	Fluoroquinolone resistance	Co-selection with β-lactams
Colistin	*mcr*	Colistin resistance	Threatens last-resort therapy
Tetracycline	*tet*	Tetracycline resistance	Historical antibiotic selection
Sulfonamide	*sul*	Sulfonamide resistance	Co-selection with other agents
Heavy Metal	*cus*, *mer*, *sil*	Heavy metal resistance	Environmental co-selection

Table 3: Summary of Key Epidemiological Studies on ESBL-Producing *E. coli*

Region	Period	Predominant Clones	ESBL Types	Key Findings
Europe	2020-2023	ST131 (C2), ST10	CTX-M-15	Human-to-human transmission predominant
North America	2021-2024	ST131 (C2), ST410	CTX-M-15, CTX-M-27	Increasing community-acquired infections
Asia (Japan)	2020-2025	ST131 (C1-M27)	CTX-M-27	Regional dominance of C1-M27 subclade
South Asia	2021-2026	ST10, ST131	CTX-M-15, CTX-M-55	High carriage; intersectoral transmission
Africa	2022-2025	Diverse STs	CTX-M-15, CTX-M-27	Genomic clusters across sectors

Table 4: Summary of Therapeutic Strategies Targeting Resistance *Plasmids*

Strategy	Mechanism	Stage	Key Challenges
CRISPR-Cas	Sequence-specific plasmid cleavage	Preclinical	Delivery efficiency; anti-CRISPR proteins;
***Plasmids* Curing Agents**	Interfere with replication/partition	Preclinical	Specificity; host toxicity;
Anti-Conjugation Molecules	Inhibit type IV secretion systems	Preclinical	Limited efficacy data;
Phage Therapy	Target plasmid-containing strains	Clinical (limited)	Host range; phage resistance;
Nanotechnology	Enhanced antibiotic delivery	Preclinical	Toxicity; manufacturing challenges;

❖ Aminoglycoside Resistance

Plasmids often carry both ESBL genes and aminoglycoside resistance genes, such as different acetyltransferases (aac), phosphotransferases (aph) and nucleotidyltransferases (ant). The presence of these determinants constrains the potential of gentamicin, amikacin and other aminoglycosides as ESBL producing organisms therapy. This high prevalence of aminoglycoside resistance genes is associated with aminoglycoside use in different eras and currently from both clinical and agricultural uses [28].

❖ Quinolone Resistance

PMQR (Plasmid mediated quinolone resistance) by the action of genes called qnr and acetyltransferase variant aac(6')-Ib-cr, has become an important issue in ESBL-producing *E. coli*. Low level resistance to fluoroquinolones is mediated through the presence of pmQR genes, which help select for high level chromosomal resistance by mutations in gyrA and parC. The fact that the PMQR genes are found together with ESBL determinants on *Plasmids* allows dissemination of resistance to two of the most important classes of antibiotics used for the treatment of Gram-negative infections, namely β-lactams and quinolones. This co-selection mechanism maintains the *Plasmids* encoding ESBLs even in the absence of beta-lactam selection when the plasmid is carried by fluoroquinolone prevalent bacteria [26].

❖ Colistin Resistance

A plasmid-mediated colistin resistance gene, mcr, is a cause for special concern in the field of antimicrobial resistance. Carbapenem-resistant Enterobacterales are often resistant to colistin, which can be a last-resort antibiotic, and the genes (mcr genes) responsible for colistin resistance have been found on *Plasmids* carrying ESBL genes and carbapenemase genes, creating strains resistant to the most critically important antibiotics. The presence of mcr on *Plasmids* which can be disseminated between One Health reservoirs (humans, animals and environment) underlines the interconnectedness in the transmission of resistance and the need for integrated surveillance [29].

❖ Tetracycline and Sulfonamide Resistance

ESBL encoding *Plasmids* are usually co-harbouring tetracycline and sulfonamide resistance genes such as tet and sul genes. Such genes are indicative of previous use of antibiotics, and of co-selection caused by the widespread use of tetracyclines and sulfonamides in human therapy as well as in agriculture. These determinants have been found to be carried on ESBL-encoding *Plasmids*, which makes the MDR phenotypes found in clinical isolates difficult to explain and reduces the potential of the agents as therapeutic options [30].

❖ Heavy Metal Resistance

The association of heavy metal resistance genes with antibiotic resistance genes in *Plasmids* is an underestimated mode of maintenance and spread of resistance determinants. *Plasmids* carrying ESBL genes often also carry genes conferring resistance to copper, mercury, silver and zinc, allowing co-selection of antibiotic resistance in heavy metal contaminated environments. This co-selection mechanism is especially important in agricultural environments where heavy metal is being used in animal feed, fertilizers and pesticides. *Plasmids* conferring heavy metal resistance in clinical strains indicate

that environmental selection forces might play a role in maintaining the presence of antibiotic resistance genes in different ecological niches [27].

Beyond Antibiotics: *Plasmids* Carry Virulence Too

❖ **Virulence *Plasmids***

Plasmids also harbor a variety of virulence determinants that give their bacterial hosts a greater pathogenic ability. Virulence *Plasmids* carry the genes for iron acquisition systems (siderophores), toxins, adhesins, capsule synthesis genes and other molecules that enable *E. coli* to cause extraintestinal infections. These virulence factors carry in *Plasmids* allow the horizontal spread of these factors, changing the commensal strain to pathogen with a single acquisition event [26].

❖ **Virulence-Resistance Convergence**

One of the most clinically important advances in the molecular epidemiology of *E. coli* infections is the commonality of virulence and the determinants for resistance on the same plasmid. Virulence genes have co-location with ESBL genes as well as carbapenemase genes within epidemic *Plasmids*, leading to highly virulent and extensively resistant strains, that are unique challenges in clinical management. This convergence can be exemplified by ST131 pandemic clone, which combines the high potential for virulence with high possibilities of spreading resistance, leading to strains with high virulence and high resistance, usually responsible for severe infections and treatment failure [26], [29]

Resistance and virulence can be acquired on *Plasmids* and such transfer is documented in a wide variety of bacterial populations with the rate of mutation or plasmid acquisition being greatly affected by host genetic background. Interestingly, most virulence genes involved in biosynthesis of capsule and lipopolysaccharide acquired mutations, primarily on the chromosome, which are associated with virulence. These structures play an important role in the formation of a biofilm, which provides an environment where *Plasmids* can be efficiently exchanged [31].

❖ **Hypervirulent MDR *E. coli***

As a result, hypervirulent MDR *E. coli* strains, which are both highly virulent and resistant to a large number of drugs, have become a clinical problem. These are found in association with serious infections such as bacteremia, meningitis and urinary tract infections and are challenging to treat because of their resistance profiles. The molecular mechanisms involved in hypervirulence and the correlation of hypervirulence with resistance *Plasmids* are subject of investigation. The discovery of hypervirulent MDR strains highlights the importance of extensive surveillance and the development of new therapeutic approaches targeting resistant hypervirulent determinants [32].

Recently, mutations in metabolic genes of hypervirulent MDR strains have shed light on the mechanisms of adaptation for long-term colonization and pathogenesis. The genetic changes in genes encoding transporters (lactose permease, dipeptide/tripeptide transporters) have been found in evolved ESBL-producing *E. coli* strains and may reflect adaptations to aid the colonizing ability of these strains in the host. Changes in metabolic genes, including sucA which codes for 2-oxoglutarate dehydrogenase, have been seen to significantly contribute to carbenicillin resistance, and changes in genes that encode parts of the response regulators, like qseB, to resistance to polymyxin B, gentamicin, and amikacin [33].

Fitness Cost Versus Fitness Compensation

❖ **Metabolic Burden**

The presence of plasmid will place a metabolic cost on the host bacteria because of the energy and resources needed for plasmid copy, maintenance and gene expression. This burden may also sometimes slow the growth rates of bacteria that have the plasmid, making them less competitive when not competing on the antibiotic. Fitness costs of the plasmid vary greatly depending on the plasmid-host pairing, as the metabolic requirement and host compensation mechanisms differ [20].

❖ **Compensatory Evolution**

A fitness cost due to plasmid carriage can be offset by compensatory mutations in the bacteria to allow for efficient plasmid maintenance in absence of selection. Compensatory evolution may take the form of mutations to chromosomal genes that enhance the compatibility of the plasmid and the host as well as mutations in the plasmid itself that lower the metabolic cost of the plasmid. Recent studies have shown that one mutational change may be enough to achieve fitness compensation, and hence bacterial adaptation to the burdens of plasmid carriage may be very efficient [20, 34, 35]

❖ **Adaptive Mutations**

Adaptive mutations that enhance plasmid-mediated fitness have been characterized in both laboratory evolution experiments and clinical isolates. These mutations can affect plasmid replication control, gene expression regulation, and host metabolic pathways, optimizing the plasmid-host relationship [36].

❖ **Long-Term *Plasmids* Maintenance**

Long term maintenance of resistance *Plasmids* in bacterial populations is dependent on a balance of costs and benefits, compensatory evolution and ecological factors. Slightly harmful fitness cost from the plasmid or fitness cost benefit from the plasmid in the absence of antibiotic selection should allow the plasmid to exist as a stable entity for long periods. Indeed, recent data indicate that, under laboratory conditions, some ESBL-encoding *Plasmids* may have a positive effect on

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the fitness of the carrier, which suggests that there may be complex interactions between plasmid carriage, host genetics and environment [20], [37]

❖ **Host Adaptation**

Plasmids can adapt to their bacterial hosts over evolutionary time, optimizing their replication, regulation, and gene content for specific host backgrounds. This host adaptation can reduce fitness costs and enhance plasmid stability, contributing to the emergence of successful plasmid-host combinations such as the IncF-ST131 association. Table 3 summarizes the fitness consequences of ESBL-encoding *Plasmids* across different host backgrounds [38].

Table 3: Fitness Consequences of ESBL-Encoding *Plasmids*

Plasmids **Feature**	**Host Background**	**Environment**	**Fitness Effect**	**Compensatory Mechanism**
IncF *Plasmids*	*E. coli* ST131	Rich medium	Low to negative	IS26-mediated deletions
IncF *Plasmids*	*E. coli* ST73	Rich medium	Higher cost	Less frequent plasmid carriage
IncN *Plasmids*	Clinical *E. coli*	Laboratory	Variable	IS-mediated restructuring
IncC *Plasmids*	Diverse STs	Laboratory	2.5-22.7% cost	Host-dependent adaptation
CTX-M *Plasmids*	Wild isolates	Enriched medium	Positive fitness	Metabolic benefits

Environmental Reservoirs of Resistance *Plasmids*

Environmental reservoirs provide a source of resistance genes that are carried by ESBL-encoding *Plasmids* and are crucial for maintaining and disseminating these *Plasmids*. Hospital wastewater is a high concentration source of antibiotic residues, resistant bacteria and resistance *Plasmids*, enabling HGT and the development of new resistance setup.

Resistant bacteria and plasmid maintenance are fostered in agricultural soil, which contains antibiotics and heavy metals from manure and pesticides. Resistance *Plasmids* can be found and spread in environments such as rivers or other water bodies that are occupied by wastewater effluents and agricultural runoff. Resistance can be transferred to humans via the food chain, especially poultry, pork and produce. *Plasmids* of resistance are also reservoirs and vectors found in animals, such as livestock and wildlife. The interface between sewage and drinking water are important entry points for humans to come in contact with environmental resistance determinants [39]. The One Health approach acknowledges the interdependence between human, animal and environmental health and offers a holistic strategy to grasp and tackle AMR. The implementation of One Health principles for ESBL-producing *E. coli* has identified unique area-specific cross-sectoral transmission patterns. In high-income countries, whole genome sequencing has shown that circulation is mostly intrasectoral with little overlap between the isolates from humans, animals and the environment. What this means is that H-to-H transmission is the main source for clinical infections in these environments, not foodborne [40].

Conversely, the studies conducted in LMICs have shown that the intersectoral transmission is often observed, with high sharing of isolates and *Plasmids* among ecological compartments. These differences are in relation to results found in the difference of sanitation infrastructure, biosecurity practices and human-animal interaction. Environmental isolates, including those from dairy farm waste, contained ESBL-producing *E. coli* clones of One Health concern, emphasizing the need for landscape-level, integrated genomic surveillance. Importance of One Health approach to region specific strategies to control AMR, which includes reducing human to human transmission in high income areas and improving sanitation/bio security in low- and middle-income areas [41].

***Plasmids*-Host Interaction**

❖ **Gene Regulation**

Plasmid-encoded genes are regulated in a way that is a reflection of the interconnectedness of plasmid and host regulatory networks. Plasmid genes are under the control of plasmid and host genes, and permit expression of resistance determinants, conjugation genes and plasmid maintenance genes to be co-regulated when responding to environmental stimuli. Host control mechanisms and plasmid gene expression have the potential to strongly impact fitness effects of plasmid carriage and spread of resistance determinants [42].

❖ **Stress Responses**

Plasmid biology can be modulated by stress responses like SOS response, general stress response and may enhance horizontal gene transfer. The SOS response can be activated by the presence of DNA damage (from antibiotic exposure, or other stress) and increases the efficiency of recombination and repair processes and can increase the efficiency of conjugation and thus horizontal gene transfer under SOS conditions. This stress-induced increase in HGT is an important pathway in the spread of antibiotic use to antibiotic resistance dissemination because antibiotic use can directly increase the spread of resistance *Plasmids* via the SOS response [43].

The impact of stress responses on plasmid biology also includes control of plasmid stability and maintenance. The environmental stress sensing to resistance gene expression has been shown to be mediated by two-component systems that include CpxR/CpxA and which regulate the expression and dissemination of β-lactamases. In strains developing resistance to the production of carbapenems, mutations of cpxA, encoding a sensor kinase, have been found, indicating that the stress response pathway may play a role in the evolution of plasmid-mediated resistance [44].

❖ ***Plasmids*-Host Coevolution**

Plasmids and their bacterial host co-evolving influences the dynamics of resistance propagation and long-term success of plasmid-host combinations. Through this process of coevolution, it is possible to minimize the fitness impact, improve plasmid stability, and maximize patterns of gene

expression, leading to the development of successful plasmid-host combinations, e.g., IncF-ST131 association. It is important to note that these large multidrug resistance *Plasmids* persist, even in the absence of antibiotic selection, in their native host cells, showing that coevolutionary adaptation results in reducing the fitness cost of the *Plasmids* [45].

The molecular basis of plasmid-host coevolution has been worked out from experimental evolution studies. In *E. coli* hosts, stability improvement of the plasmid can occur easily by IS26-mediated deletion of the less desirable parts of the plasmid backbone, essentially broadening the host range of the plasmid. An evolutionary strategy which is consistently used by evolved *E. coli* lineages shows that there is a trade-off between horizontal and vertical transmission, which could constrain dissemination potential of clinically multidrug resistance *Plasmids*. The function of insertion sequences in promoting the plasticity of the plasmid is essential in the process of overcoming plasmid-host constrains and . . . in adaptation to new kinds of bacteria [46].

Cutting-Edge Technologies for *Plasmids* Characterization

- **Sequencing Technologies**

While short-read sequencing can give accurate base calling and high coverage, it cannot resolve repetitive regions or complex plasmid structures especially for Illumina. Long read sequencing technologies like Oxford Nanopore and PacBio can produce reads covering the entire plasmid, allowing complete plasmid assembly and characterization of structural variability. The use of hybrid assembly (short reads + long reads) results in the best possible quality plasmid sequence; these methods combine the best of both short reads and long reads [47].

- **Advanced Genomic Technologies**

Optical mapping, Hi-C sequencing, single-cell genomics, and metagenomics provide complementary approaches to plasmid characterization. Plasmidomics, the systematic analysis of plasmid populations, has emerged as a field focused on understanding plasmid diversity, distribution, and dynamics. Artificial intelligence (AI)-assisted plasmid prediction tools are enhancing our ability to identify resistance *Plasmids* in genomic and metagenomic datasets [48].

Artificial Intelligence and Machine Learning in Resistance Prediction

The use of artificial intelligence (AI) and machine learning in predicting resistance is currently a frontier field, and is likely to play a larger role in the future of AMR surveillance and decision support in clinical practice. Convolutional neural networks, recurrent neural networks and other deep learning methods can detect patterns in genomic and metagenomic data that can predict resistance phenomes with high accuracy [49].

Genomic, epidemiological, and environmental information can be combined into predictive epidemiology models to predict emergence and spread of resistance and guide proactive management. Machine learning can predict future resistance trends – resistance forecasting can be used in antibiotic stewardship and policy making. AI-based classification of *Plasmids* according to their genetic characteristics contributes to an understanding of the epidemiology and evolution of *Plasmids*. Machine learning based genome mining can predict new (potential resistance) genes and plasmid in Genomic data to foresee the upcoming threats even before they gain clinical importance [50].

Therapeutic Strategies Targeting Resistance *Plasmids*

- **CRISPR-Cas Systems**

CRISPR-Cas systems are a potential approach in combating resistance *Plasmids* with sequence-specific cleavage and removal of plasmid DNA. CRISPR-Cas systems like CRICON can be used to lower the incidence of ESBLs in bacterial communities by using conjugatively delivered systems. The effectiveness of the CRISPR targeting antimicrobials, however, depends on a range of ecological and evolutionary factors, such as the efficiency of conjugation, the fitness costs imposed on the plasmid, the mobility of the plasmid and the ability to develop resistance to the CRISPR targeting sequence. CRISPR in complex microbial communities is challenged by bacterial defense mechanisms and anti-CRISPR proteins, and because of target site mutations [51].

- ***Plasmids* Curing Agents**

The chemical approach to reversing resistance is through the use of plasmid curing agents, such as small molecules that selectively cure *Plasmids*. These agents act on plasmid replication, partition, and/or maintenance causing plasmid loss and recovery of antibiotic susceptibility [52].

- **Anti-Conjugation Molecules**

Molecules that inhibit conjugation, including inhibitors of type IV secretion systems and compounds that disrupt mating pair formation, can block horizontal gene transfer and limit resistance dissemination [53].

- **Phage Therapy**

Bacteriophages, viruses that infect bacteria, can target plasmid-containing strains, providing a biological approach to reducing resistance prevalence. Phage therapy has been explored for the treatment of infections caused by ESBL-producing *E. coli*, with promising results in some cases [54].

- **Nanotechnology**

Nanotechnology-based approaches, including antimicrobial nanoparticles and drug delivery systems, offer novel strategies for combating plasmid-mediated resistance. Nanoparticles

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can enhance antibiotic efficacy, target resistance *Plasmids*, and deliver antimicrobial agents to infection sites [55].

❖ **Antisense Oligonucleotides**

Antisense oligonucleotides, which bind to and inhibit plasmid-encoded mRNAs, can silence resistance gene expression and restore antibiotic susceptibility [56].

❖ **Synthetic Biology**

Synthetic biology approaches, including the engineering of bacterial strains that outcompete resistant strains or that degrade resistance *Plasmids*, offer innovative strategies for AMR control [57].

❖ **Gene Editing**

Gene editing technologies, including CRISPR-Cas systems and other programmable nucleases, can specifically edit resistance genes, eliminating resistance while preserving bacterial viability [58].

Discussion

The literature presents some apparent discrepancies that demand careful study. Regarding transmission patterns, high-income nations reveal largely intrasectoral circulation with low overlap between human, animal, and environmental isolates [11, 40]. This data contradicts notions regarding foodborne transmission as a main cause of clinical ESBL infections in these settings. Conversely, low- and middle-income nations typically uncover similar genetic clusters across all one health compartments [12, 13, 41], highlighting disparities in sanitation infrastructure and human-animal interactions. Regarding plasmid fitness costs, whereas early studies highlighted metabolic load [20], current research indicates great diversity. IncF *Plasmids* in ST131 contexts show modest fitness costs, but the identical *Plasmids* in ST73 backgrounds impose larger costs [38]. Some CTX-M *Plasmids* appear to provide favorable fitness effects in enriched medium [37]. This context-dependence has major consequences for resistance persistence and challenges efforts to eliminate resistance through antimicrobial stewardship alone.

However, several knowledge gaps exist that hamper a complete understanding of and effective intervention against the spread of ESBL-encoding *Plasmids*; (i) the molecular determinants of successful plasmid-host combinations are poorly characterized; (ii) the genetic basis of fitness compensation is incompletely understood; (iii) the role of environmental reservoirs in maintenance of ESBL-encoding *Plasmids* needs further quantification [39]; and (iv) AI-assisted resistance prediction models face limitations such as reliance on non-representative training datasets and difficulty predicting novel resistance mechanisms [49, 50].

Figure Description

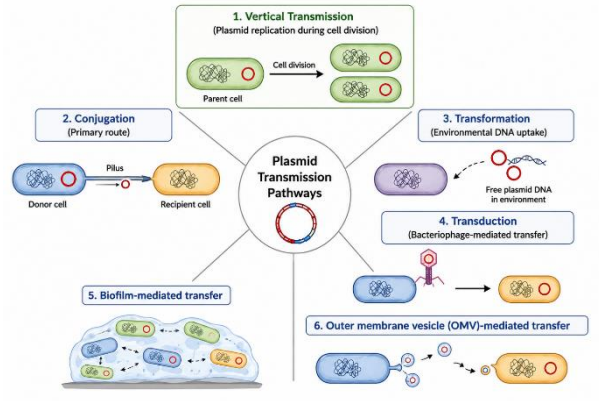

Figure 1. Major pathways of plasmid-mediated dissemination of ESBL-associated antibiotic resistance.

Schematic representation of the main strategies for dissemination of ESBL-encoding *Plasmids* in bacterial populations. The figure shows vertical transmission via plasmid replication during bacterial cell division and horizontal transmission by conjugation (the main mechanism), transformation (uptake of extracellular plasmid DNA), transduction (bacteriophage-mediated transfer), biofilm-mediated plasmid exchange and outer membrane vesicle (OMV)-mediated transfer. Together, these pathways facilitate the rapid dissemination and colonization of antimicrobial resistance genes among multiple bacterial species and ecological niches.

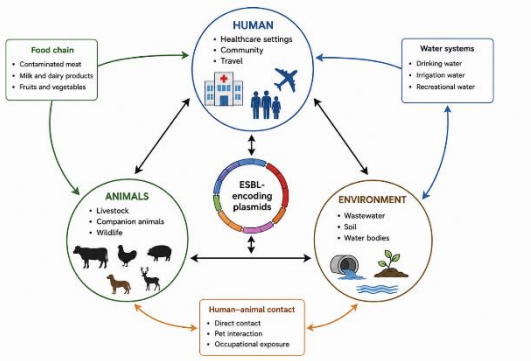

Figure 2. One Health transmission model of ESBL-encoding *Plasmids*.

Conceptual framework illustrating the interconnected transmission of ESBL-encoding *Plasmids* between human, animal and environmental compartments in the One Health continuum. Human sources include health care, community and international travel. Animal reservoirs include livestock, companion animals and wildlife. Environmental reservoirs, including wastewater, soil and water bodies, act as a reservoir for resistant bacteria and *Plasmids*, allowing their persistence and spread. Key transmission interfaces include the food chain, water systems and direct human–animal contact, which enable the bidirectional transfer of determinants of antimicrobial resistance and contribute to the global spread of ESBL-producing bacteria.

Conclusion

Plasmids are the major players of the global epidemic of ESBL-producing *E. coli* and act as vehicles for the transfer of resistance genes between species, sectors and geographic regions. The complexity of plasmid biology, from control of replication to their means of propagation to their interactions with the host, implies a long evolutionary history and adaptation to a variety of environmental niches. The *Plasmids*, which are capable of sequestration of resistance determinants, co-localization of virulence determinants and adaptability to new host by fitness compensation, have enabled a highly successful MDR clone like ST131 to emerge.

Such epidemic *Plasmids* often harbor both resistance and virulence determinants, which have led to the creation of MDR pathogens of particular clinical relevance that pose a threat to therapeutic options and public health interventions. Advances in genomic techniques such as long-read sequencing and AI-powered analysis have shed new light on the epidemiology of *Plasmids*, uncovering a complex landscape of dissemination patterns that differ across regions and ecological niches.

The plasmid-mediated resistance crisis requires a One Health approach with multiple surveillance systems that incorporate human, animal and environmental aspects of this problem. New therapeutic approaches to combat resistance *Plasmids* such as the use of CRISPR-Cas, anti-conjugation molecules and phage therapy give hope to limit their effects.

Several areas of critical gaps of knowledge are still unanswered, such as the reason why some plasmid-host combinations work, molecular mechanisms of fitness compensation, and the potential role of AI to predicting resistance emergence. These gaps will need to be filled through ongoing investments in basic research, surveillance, and innovative interventions. Plasmid mediated resistance is a key problem today in the context of infectious diseases and demands collective efforts at the international level to ensure that the effectiveness of antibiotics is preserved for the benefit of future generations. Plasmid biology needs to be understood as a whole, combined with systems-level approaches and One Health surveillance, if we are to come to grips with the emerging problem of ESBL-producing *E. coli* and maintain the progress of modern medicine.

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Authors' Contribution:

- All Authors Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

Correspondence:

Aqsa Batool

aqsakazmi@ymail.com

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