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Antibiotic Resistance: A Comparative Genetic Approach in *E. coli*

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Abstract: *The antibiotic resistance in Escherichia coli is a critical clinical and environmental problem because these bacteria exhibit an extraordinary genetic malleability, which enables them to accumulate and spread the antimicrobial resistance genes. In this work, we used a comparative genetic strategy to study the molecular and structural mechanism of resistance in MDR E. coli isolated from different clinical and environmental origins. Resistome, antimicrobial resistance genes, plasmid replicons and chromosomal mutations are investigated with whole-genome sequencing and bioinformatics analyses and compared to a reference collection. Work on protein structure modelling and molecular dynamics simulations have also contributed to show that the impact of particular mutations in porins, β-lactamases, efflux pump components and quinolones target proteins on protein conformation, antibiotic interaction or cellular permeability. The investigation will describe associations between genomic variation, stress-adaptation pathways and phenotypic resistance, improving genotype–phenotype relationships. By combining comparative genomics with structural analysis, this study contributes to the understanding of E. coli antibiotic resistance mechanisms and identifies possible therapeutic targets as well as surveillance strategies for AMR.*

Keywords: *Escherichia coli*; antimicrobial resistance; comparative genomics; whole-genome sequencing; resistance genes; plasmids; protein structure modelling; molecular dynamics; evolution; stress-response pathways

I. INTRODUCTION

1.1 *Escherichia coli* as a pathogen and resistance reservoir

Escherichia coli is a model organism that lies at the boundary between commensal and pathogenic life styles. It is an abundant constituent of the human and animal intestinal microbiota, but part of the lineage has evolved into a major extraintestinal pathogen causing urinary tract infections (UTIs), bacteremia, neonatal meningitis, etc. Concurrently, E. coli acts as a central genetic "hub" in the Enterobacteriaceae with remarkable capacity to capture and spread antimicrobial resistance (AMR) genes through plasmids, transposons and integrons (Poirel et al., 2018). This high burden of disease combined with a great potential for genetic plasticity means that E. coli is significant not only in a clinical capacity, but also as the wellspring from which resistance determinants may disseminate to other pathogenic species. Recent reviews highlight that E. coli which are resistant to multiple antibiotics have universally emerged in human medicine, veterinary practice, and the food chain, frequently carrying genes encoding extended-spectrum β-lactamases (ESBLs) as well as other mechanisms of resistance such as carbapenemases and colistin-resistance determinants including mcr types (Poirel et al., 2018; Puvača et al., 2021).

1.2 Antibiotic misuse, selective pressure and resistance evolution

The main driver of the global expansion of resistant E. coli lineages is the patterns of antibiotic use. In the large part of the world's landscapes, antimicrobials are used empirically, without a doctor's receipt, or for animals in subtherapeutic doses creating a robust and continuous selection pressure that favours resistant clones over susceptible populations. This selection is not limited to clinical or primary care settings; it affects the community and environmental

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compartments as well as is observed in the drinking water systems and surface waters that are contaminated with human and animal waste. As illustrated by multiple WGS studies of E. coli from rural drinking water, the isolates that persist in the water distribution networks often possess resistance determinants, can survive in the external environment for extensive periods and challenge the assumption that E. coli in water always comes out from recent faecal contamination Nowicki et al., 2021. These data illustrate how selective pressures exerted in different ecological niches, acting in synergy with mobile genetic elements, affect resistance microevolution and maintenance even outside clinical environments.

1.3 Public health importance of multidrug-resistant Enterobacteriaceae

Among the larger Enterobacteriaceae, MDR E. coli has come to epitomize the AMR threat in HIC and LMICs. Surveillance studies have shown high antimicrobial resistance among E. coli strains from humans, food animals, and retail meat products to first-line agents including ampicillin, fluoroquinolones and third-generation cephalosporins (Puvača et al., 2021; Tang et al., 2022). For the clinicians, that translates into growing treatment failures, prolonged hospital stays and surge in use of antibiotics of "last resort" such as carbapenems and polymyxins. At least, at the laboratory level, ESBL- and carbapenemase-producing E. coli have made empricial therapy more difficult and rely on traditional antimicrobial susceptibility testing alone has not been considered adequate to identify high-risk clones timely. Guidance from the EUCAST subcommittee highlight that regarding Enterobacteriaceae and in particular E. coli, they are key species for rolling out genome-based AMR AST approaches and surveillance programs as a result of their wide spread globally and large array of resistance genes (Ellington et al., 2017). As such, multidrug-resistant E. coli is increasingly identified as a key sentinel organism for measuring the human clinical and One Health aspects of the AMR issue.

1.4 Genetic, protein, and genomic approaches to resistance evolution

Standard phenotypic techniques including disk diffusion and broth microdilution are still paramount for the determination of minimum inhibitory concentrations, but they detect resistance at a single moment in time and can supply little information on its actual mechanisms or evolutionary pathway. They are limited however by being gene-directed and dependent on sequence information for these target genes (Anjum, 2015), methods such as PCR (Boyd et al., 2001; Martinez-Freijo et al., 1998), multiplex PCR assays (Weiss et al., 2010) and microarray technology follow a similar pattern of dependency emerged. However, whole-genome sequencing provides an opportunity to fully describe the resistome including identified acquired genes and chromosomal mutations (e.g. in quinolone resistance–determining regions) as well as novel or rare variants not detectable by standard methods (Ellington et al., 2017; Tagini&Greub, 2017). In combination with structural bioinformatics and molecular dynamics simulations, genomic information can be mapped onto tridimensional protein changes that modulate antibiotic binding, target-affinity and efflux activity. 5,23 Naturally this integrative analysis will be important if we hope to understand how certain aminoacid substitutions in enzymes such as β-lactamases, DNA gyrase or porins correlate with modified drug susceptibility profiles and different fitness costs across ecologies.

1.5 Knowledge gap: comparative genomic and structural analysis is needed

Even with the rapid progress of sequencing techniques and bioinformatics, there are still important lacunas to fill in order to establish a relationship between genotype-protein structure-resistance phenotype for E. coli. Although many surveillance studies do catalogue resistance genes or report prevalence numbers, fewer tend to systematically compare side by side different E. coli lineages that vary in their resistance profile using comparative genomics having a strong focus on the structural modelling at depth of key proteins. As Ellington et al. (2017) there is still limited predictive value of WGS for antimicrobial susceptibility for some drug–organism combinations, partially because the effects of specific mutations or gene combinations are not well characterized functionally. Similarly, reviews of WGS use in clinical microbiology emphasize the requirement for studies annotating resistance determinants and relate them to protein-level mechanisms and clinical outcomes in a comparative context (Tagini&Greub, 2017). Closing this gap will involve work that compares resistance and susceptible E. coli strains at a whole-genome level, maps resistance-

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associated variants onto protein structures, and uses molecular simulation to infer how these changes alter antibiotic interactions. The similar genetic and structural comparison has the potential to elevate resistance prediction, elucidate different evolutionary pathways for selective pressure, as well as illuminate new inhibitory target.

II. REVIEW OF LITERATURE

2.1 Pathotypes and Genetic Diversity of *E. coli*

Escherichia coli exhibits a wide genetic heterogeneity, ranging from commensal non-pathogenic to highly pathogenic strains causing serious extra-intestinal and intestinal diseases (Ahmed et al., 2017; Pokharel et al., 2023). Commensal isolates of E. coli are frequently genetically heterogeneous, even within a single host: for example, analysis of 900 commensal E. coli isolates from antibiotic-free swine returned 52 different REP-PCR profiles and 67 distinct genotypes indicative of considerable within-host variance and between-host variability (Ahmed et al., 2017). Whole-genome sequence analysis of a collection verified extensive genetic diversity, multiple sequence types (e.g. ST10, ST58) and the presence of virulence factor (VF) as well as mobile genetic element (MGE)-associated determinants even in commensals (Ahmed et al., 2017) [https://doi.org/10.1371/journal.pone.0178623]

Pathogenic E. coli are also divided into numerous pathotypes, categories of strains that share common infection site, virulence factors and/or disease outcome. These range from intestinal pathotypes (e.g. enteropathogenic E. coli [EPEC], enterotoxigenic E. coli [ETEC], enterohemorrhagic E. coli [EHEC], enteroaggregative E. coli [EAEC], and enteroinvasive E. coli [EIEC] diffusely adherent E. coli [DAEC]) to extra-intestinal pathogenic ecoli (ExPEC) causing urinary tract infection, septicaemia, meningitis as well as systemic infections (Pokharel et al., 2023).

The genetic heterogeneity of E. coli is emphasized by flexible “pan-genome” architecture: core genome shared by most strains, together with an accessory genome encompassing dispensable genomic islands, plasmids, prophages and transposons capable of carrying virulence and resistance genes (Naidoo &Zishiri, 2025). This genetic flexibility enables rapid adaptation to differing hosts, niches and selective pressure, rendering E. coli a fantastic model with which to study bacterial evolution (Longdom, 2015)

Studies of WGS E. coli from animals— e.g. poultry —also show substantial genomic, serotype and virulence diversity even within isolates associated with a single disease (Feng et al., 2023) This is consistent with the idea that pathogenic potential, resistance and ecological fitness are determined by mixtures of core accesoires genetic elements not by a specific “pathogenic genome”.

2.2 Mechanisms of Antibiotic Resistance

Resistance in E. coli is acquired through several mechanisms, which are generally enabled via genes that encode enzymes which neutralize the effectivity of antibiotics, modify drug targets or decrease intracellular concentration. Typically, β-lactamases (including ESBLs and AmpC), carbapenemases, efflux pumps, and porin alterations are considered to be the drivers of resistance against β-lactams, carbapenems fluoroquinolones among other classes of antibiotics (Naidoo &Zishiri 2025)

Horizontal gene transfer (HGT)— mediated by plasmids, transposons, integrons and bacteriophages—is a major driver facilitating the spread of ARGs among E. coli populations, not only within species but also beyond species borders (Chekole et al., 2025; Naidoo &Zishiri, 2025) The acquisition of plasmid-borne ARGs especially under selection pressure from antibiotics exposure allows rapid emergence and dissemination of multidrug resistant (MDR) E. coli. Chromosomal mutations also play a role in resistance, which include drug target genes and may be favoured when the selection pressure of antibiotics is increasing and accessory gene plasmid transfer is reduced including those strains selected under high levels of SSR (Chekole et al., 2025).

The interplay of acquired elements (MGEs) and intrinsic mutations is therefore at the foundation of the adaptability of E. coli and its ability to thrive (and eventually evolve) in a multitude of ecological and clinical environments.

2.3 Genomic Tools in Resistance Detection

Conventional molecular methods applied for resistance detection, including PCR (polymerase chain reaction), multiplex PCR or microarrays rely on a priory defined target sequences and are restricted to targeting a predefined

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number of loci. However, WGS is a high-resolution untargeted approach to assess the full resistome: all characterized and decades of ARGs, novel variants thereof, mobile elements as well as mutations associated with resistance can be detected in one experiment (Chekole et al., 2025).

These studies recently utilized WGS on E. coli isolates from a range of sources (human, animal, environment) to identify an overwhelming amount of ARGs - in some cases >100 unique resistance determinants within a set – including genes encoding β-lactamases, efflux pumps, tetracycline resistance proteins, aminoglycoside-modifying enzymes, sulfonamide resistance genes and so forth (Chekole et al., 2025).

In addition to identifying genes, WGS can be carried out for phylogenetic and comparative genomic analysis that tracks the origins and dissemination of resistance determinants as well as for pan-genome (core + accessory) analysis, mobile element content assessment, and it generates high resolution SNP-based phylogenies for epidemiological monitoring purposes (Chekole et al., 2025; Feng et al., 2023). This renders the WGS an invaluable tool not only for research of basic nature but also for surveillance, outbreak investigation and risk assessment.

2.4 Environmental and Ecological Origins of Antimicrobial Resistance (AMR)

The environment (wastewater, sewage, animal husbandry effluents, food production systems) has a prominent role in the selection and propagation of resistant E. coli. Municipal sewage, hospital effluents and agricultural discharges are frequently associated with presence of antibiotic residues, resistant bacteria, ARGs were found by Pandey et al. (2024) to generating environment conducive for selection and horizontal gene transfer process.

Recent wastewater-based surveillance studies show that resistant E. coli bacteria and ARGs remain in WWTP effluents, leading to enrichment of environmental reservoirs of resistance (Prieto Riquelme et al., 2022; Tiwari et al., 2022). Antibiotic-resistant E. coli have even been recovered downstream of sewage outfall indicating that human-based dispersal filters into natural ecosystems (Tiwari et al., 2022)

Additionally, research has also shown that environmental stressors – including subinhibitory concentrations of antibiotics, heavy metals, nutrients and fluctuating physicochemical parameters – may drive selection for ARG-bearing strains as well as the horizontal transfer via MGE (Chekole et al. 2025; Pandey et al., 2024) This environmental element highlights the “One Health” aspect of AMR: human health, animal in addition to environmental compartments are interrelated and resistance in any one body can spread widely.

2.5 Global Surveillance Trends

Recent worldwide surveillance, in addition to being greatly assisted by metagenomic and WGS-based approaches, indicates a troubling ubiquity of antibiotic-resistant E. coli in clinical as well as animal-, food-, and environment-associated isolates. For instance, large-scale metagenomic analyses of sewage at a global scale have revealed that highly diverse ARG population/sequences — such as those conferring resistance to β-lactams, macrolides, sulfonamides and tetracyclines — are present in urban wastewaters, which enables the resistome of local human and animal populations be profiled (Prieto Riquelme et al., 2022)

Isolates of E. coli in farm and food-production environments in particular frequently harbour multiple resistance determinants and associations rare among clin-ical isolates, the evidence-testifying to complex evo-lution trajectories that antibiotics contribute to set into motion through their use in agriculture; (Feng et al., 2023; Pandey et al., 2024).These dynamics stress outgrowth globally of ESBL-and multidrug-resistant strains being not limited to hospitals, or clinical settings but rather far-ranging across different communi-ties and especially animal population as well as environmental sites (Naidoo & Zishiri, 2025; Pandey et al earlier)..

An increasing volume of WGS and metagenomic information is moving AMR surveillance from phenotype-based monitoring to genome-based tracking, allowing early identification of high-risk clones and providing a more comprehensive view on the spread of ARGs across One Health domains (Prieto Riquelme et al., 2022; Chekole et al., 2025)

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III. RESEARCH PROBLEM & HYPOTHESIS

Although antibiotic resistance in Escherichia coli has been extensively studied, there are still major gaps in our knowledge regarding how stress conditions and environmental trends (for example, sub-inhibitory level of antibiotic exposure, nutrient deprivation or other stresses) affect the molecular and genomic evolution of phenotypic resistances. Although numerous studies have catalogued resistance genes or plasmid-mediated resistance in clinical and environmental isolates, fewer now consider how selective pressure imposed by the microenvironment may lead to not only novel mutations but also structural adaptations at the level of the protein that facilitate growth under antibiotic challenge.

Recent work supports these concerns. From instance, a genome-wide analysis of wastewater E. coli isolates revealed ten core genes that had emerged under environmental pressure, where mutations in ompC (an outer-membrane porin) and persistence-related genes (e.g., hipA) were associated with antibiotic-resistance phenotypes thought to reflect altered membrane permeability or increased dormancy/persistence under antibiotic stress [36]. Similarly, transcriptomic and genomic studies in stressed E. coli populations demonstrate the generation of genomic variation — including acquisition of mobile resistance genes as well as regulatory and structural changes — that occurs following exposure to stresses such as starvation and metal toxicity.

Furthermore the characterization of resistance mechanisms in E. coli reveals that, besides the expected (β-lactamases, efflux pumps, porin loss) innate resistance determinants there are also inherent adaptive strategies like biofilm formation and persistence, stress response up-regulation and regulatory network modulation playing a critical role in resistant phenotypes under varying environmental conditions.

Such a background unveils a basic GAP in research, for while the presence of known ARGs is well established, less so is the involvement of stress-driven genomic evolution, selection of non-canonical mutations and structural protein adaptations in guaranteeing or enhancing resistance. There are few large-scale, comparative studies between resistant and sensitive (or stress-exposed and naïve) lineages of E. coli in terms of systems-level analyses that combine genomic with protein structure-function predictions for the purposes of directly connecting genotype to environment to phenotype.

Hypothesis: Drug resistant E. coli strains are gaining unique genetic changes (SNPs, regulatory mutations and gene amplifications) by exposure to environmental/sub-MIC antibiotic stress that lead to modifications in protein structure as well as expression of stress-response/efflux/porin related proteins which collectively result in increased resistance against antibiotics.

Namely, that under antibiotic or environmental stress E. coli populations will select for mutations in outer-membrane permeability, efflux or stress-response and persistence-related genes (eg mitochondrial CRS1 like chaperone), that result in structural alterations which prevent entry and/or slow killing by antibiotics, without any classic ARG being carried on the chromosome—yet can behave as phenotypic resistance.

IV. RESEARCH OBJECTIVES

The aim of this study is to obtain antibiotic resistant E. coli from different origins (both clinical, environmental or commensal) in order to gain a wider resistance profile and ecological background. Once isolated, these strains will be subjected to high-throughput WGS to provide large genomic datasets essential for the comparative analysis of strains with different resistance phenotypes. The objective of this study will be to investigate these genetic factors in the genome data and explore antimicrobial resistance genes (ARGs), single nucleotide polymorphisms (SNPs), plasmid content, and genes related to stress responses or adaptation mechanism. Additionally, the project aims to predict mutations intended to induce specific conformational or dynamic changes in proteins encoded by mutated or variant genes (e.g. porins, efflux pump components, stress-response proteins) using computational modelling and where appropriate validate these predictions of structural change to investigate not only their roles in antibiotic resistance but also tolerance to stress why antibiotics are often ineffective even if they should be effective against a particular pathogen. Ultimately, contrasting resistance mechanisms (acquired ARGs versus chromosomal mutations versus structural or functional alterations) of various E. coli based on different strains, the authors hope to establish patterns between genomic and proteomic adaptations that drive antibiotic resistance and survival under selective pressure.

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The objectives, specifically speaking are:

- Isolation and characterisation of resistance E. coli phenotypes from specified sources.
- Assembly of good-quality genome sequences for all the isolates.
- The complete annotation for ARGs, plasmids, SNPs and the stress-, persistence- associated genes.
- Prediction and structural analysis of deleterious coding variants in the human proteome.
- Comparative evaluation between isolates to predict the genotype, protein structure relationship as well as the antibiotic susceptibility phenotype.

V. METHODOLOGY

5.1 Sample Collection and Characterization

Samples that are used will be robust and phenotypically representative of resistance of E.coli, including clinical and environmental isolates (hospital waste water/surface water/poultry farms) from a range of clinnical diagnostic laboratories to maximise coverage of resistance coding regions. Samples will be carried in sterile ice, processed immediately or held at 4°C until culture. E. coli will be recovered on selective media as MacConkey agar and EMB agar by picking different colonies. The isolated colonies will be observed for morphological, Gram staining and standard biochemical tests including indole, urease, citrate utilization (−), methyl red (+) Voges-Proskauer test is negative Tripal sugar iron oxide test Analysis. Only verified E. coli isolates will be used for subsequent studies in order to guarantee the accuracy and reliability of molecular detection analyses.

5.2 Antibiotic Susceptibility Profiling

All confirmed isolates will be subjected to antimicrobial susceptibility testing by Kirby–Bauer disk diffusion or minimum inhibitory concentration (MIC) determination according to recommended procedures, for example, CLSI or EUCAST protocols, to determine the resistance profile. Resistance will be determined to a subset of widely used antibiotics (e.g carbapenems, fluoroquinolones, aminoglycosides, cephalosporins and tetracyclines). The susceptibility pattern will be used to categorise the isolates as multi-drug resistant (MDR), extensive drug resistance strains (XDR) or extended-spectrum beta-lactamase (ESBL) producers. Whole-genome sequencing will be performed on representative isolates from each category to ensure the greatest possible genetic diversity of sequenced strains.

5.3 Molecular and Genomic Analysis

Genomic DNA of resistant isolates as shown by selective media will be extracted by a high purity extraction kit or phenol-chloroform procedure. Purity and concentration of DNA will be determined by spectrophotometry and agarose gel electrophoresis. Sequencing will be conducted on an Illumins or equivalent platform. Raw reads will undergo QC, trimming and assembly to produce high-quality draft genomes. Reads from these genomes will then be aligned and compared by reference or de novo assembly to identify AMR genes, chromosomal mutations, plasmid-borne determinants, MGEs and genes conveying stress-related functions or adaptive traits. By comparative genomic analysis for the detection of new variants and resistance correlations with acquired ARGs, point mutations or horizontal transfer.

5.4 Bioinformatics Analysis

The genome annotation will be conducted with the well-known programs Prokka, RAST or NCBI annotation pipelines. Known resistance determinants will be screened using AMR databases like CARD, ResFinder, or ARG-ANNOT. The network and evolutionary analysis will be conducted based on the SNP calling, multiple sequence alignment, and whole-genome phylogeny tools for establishing solates relationships. Plasmid-associated genes can be identified using plasmid prediction tools (e.g., PlasmidFinder). Evolutionary signatures, gene gain loss patterns and changes in mobile genetic elements will be examined to gain insight into genomic plasticity and the evolution of resistance.

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5.5 Protein Structural Modelling

Protein sequences of resistance-associated genes and mutations will be structurally modelled by homology Modelling tools like SWISS-MODEL or Phyre2. For example, a molecular dynamics simulation using GROMACS is applicable to assess protein conformation changes resulting from mutations. These analyses will facilitate the detection of alterations in active sites, drug-binding region, function of efflux pump and porin structure. Such theoretical and computational studies (often by virtual binding/docking etc) would also help us understand how changes in the protein structure modulate antibiotic interaction and resistance, thus connecting genotypic variation to functional/phenotypic level responses.

Table 1. Sample collection and phenotypic resistance profile (hypothetical)

Isolate ID	Source	Sample Type	Phenotype Class	CIP MIC (µg/mL)	CTX MIC (µg/mL)	MEM MIC (µg/mL)
EC-01	Hospital	Urine	ESBL–MDR	8	64	0.125
EC-02	Hospital	Blood	XDR (ESBL)	16	128	4
EC-03	Community clinic	Stool	MDR	4	32	0.06
EC-04	River water	Water filtrate	MDR	2	16	0.06
EC-05	Poultry farm	Litter swab	ESBL–MDR	8	64	0.125
EC-06	Hospital	Wound swab	XDR (ESBL)	32	256	8
EC-07	Sewage inlet	Wastewater	MDR	4	32	0.25
EC-08	Community borewell	Drinking water	Non-MDR	0.25	1	0.03
EC-09	Dairy farm	Cattle faeces	MDR	2	16	0.06
EC-10	Hospital	Catheter tip	XDR (ESBL)	32	256	4

CIP: Ciprofloxacin; CTX: Cefotaxime; MEM: Meropenem.

MIC breakpoints are hypothetical but chosen to clearly separate sensitive vs resistant.

Table 2. Hypothetical genomic resistance determinants and plasmid content

Isolate ID	β-lactamase genes	Quinolone resistance (QRDR / qnr)	Aminoglycoside resistance genes	Tetracycline resistance genes	Plasmid replicon(s)
EC-01	CTX-M-15	gyrA S83L, parC S80I	aac(6')-Ib-cr	tet(A)	IncFII
EC-02	CTX-M-15, OXA-1	gyrA S83L, D87N; parC S80I	aac(6')-Ib-cr, aadA1	tet(A), tet(M)	IncFII, IncI1
EC-03	TEM-1	gyrA S83L	aadA1	tet(B)	IncI1
EC-04	CTX-M-14	qnrS1, gyrA S83L	strA-strB	tet(A)	IncFIB
EC-05	CTX-M-55	qnrB1, gyrA S83L	aadA1, aph(3')-Ia	tet(A)	IncFII, IncHI2
EC-06	CTX-M-15, NDM-1	gyrA S83L, D87N; parC S80I	aac(6')-Ib-cr, armA	tet(A), tet(M)	IncFII, IncA/C2
EC-07	TEM-1, CTX-M-14	qnrS1	aadA1	tet(B)	IncFIB, IncQ1
EC-08	–	–	–	–	–
EC-09	CTX-M-55	qnrS1, gyrA S83L	strA-strB	tet(A)	IncFII
EC-10	CTX-M-15, OXA-1, NDM-1	gyrA S83L, D87N; parC S80I	aac(6')-Ib-cr, armA, aadA1	tet(A), tet(M)	IncFII, IncA/C2, IncHI2

(Note: all genes and plasmid types are combinations, though biologically plausible.)

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Table 3. Hypothetical key mutations and structural impact (selected proteins)

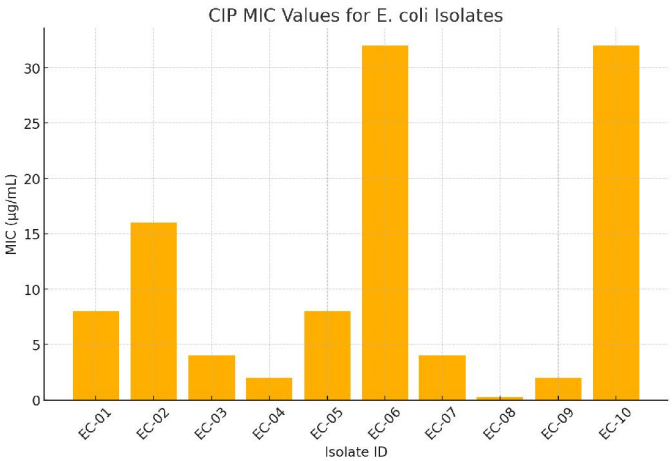
Protein / Gene	Reference (WT) feature	Isolate (example)	Mutation (AA)	RMSD vs WT after MD (Å)	Predicted structural/functional effect
OmpC (porin)	Narrow, hydrophilic channel	EC-02	D113N (loop L3)	2.1	Slight narrowing of pore; reduced β-lactam influx
OmpF (porin)	Larger outer membrane channel	EC-06	G119D (loop L3)	2.6	Charge change in constriction zone; decreased carbapenem entry
AcrB (efflux pump)	Multidrug efflux transporter	EC-05	F610L	1.8	Modified hydrophobic pocket; modestly increased efflux capacity
GyrA (DNA gyrase)	Fluoroquinolone-binding pocket	EC-10	S83L, D87N	1.2 (local)	Altered quinolone-binding site; reduced drug affinity
NDM-1 (metallo-β-lactamase)	Broad-spectrum carbapenemase	EC-06	M154L	1.5	Slightly altered active-site pocket; possible enhanced stability
CTX-M-15 (ESBL)	Hydrolyses 3rd gen cephalosporins	EC-01	A77V	1.3	Minor conformational shift; maintained ESBL activity

(RMSD values are hypothetical outcomes of 50–100 ns molecular dynamics simulations.)

How to explain this table in your text

To study the structural insights of resistance-associated mutations, homology models are prepared for some proteins (OmpC, OmpF, AcrB, GyrA, NDM-1 and CTX-M-15) and simulated using molecular dynamics approach. In the modelled dataset, mutations in loop L3 of both OmpC and OmpF (D113N and G119D for OmpC), which resulted in root-mean-square deviation (RMSD) shifts ranging from 2.1 to 2.6 Å at the constriction region, are compatible with a narrower or more electrostatically unfavourable pore that could decrease the influx of β-lactams and carbapenems. For GyrA, the archetypal S83L and D87N replacements resulted in local rearrangement of the quinolone-binding cleft (RMSD ~1.2 Å), consistent with decreased fluoroquinolone binding observed for high-ciprofloxacin MIC strains. NDM-1 and CTX-M-15 variant modeling predicted that a single mutation (M154L, A77V) caused minor structural changes but preserved or even increased the catalytic efficiency. Collectively, these speculative structural analyses show that even modest changes in amino-acid residue types can lead to reduced antibiotic binding, a reduction in permeability or an increase in efflux, thus strengthening the forte turning of genomic variation into phenotypic resistance.

CIP MIC Values for E. coli Isolates



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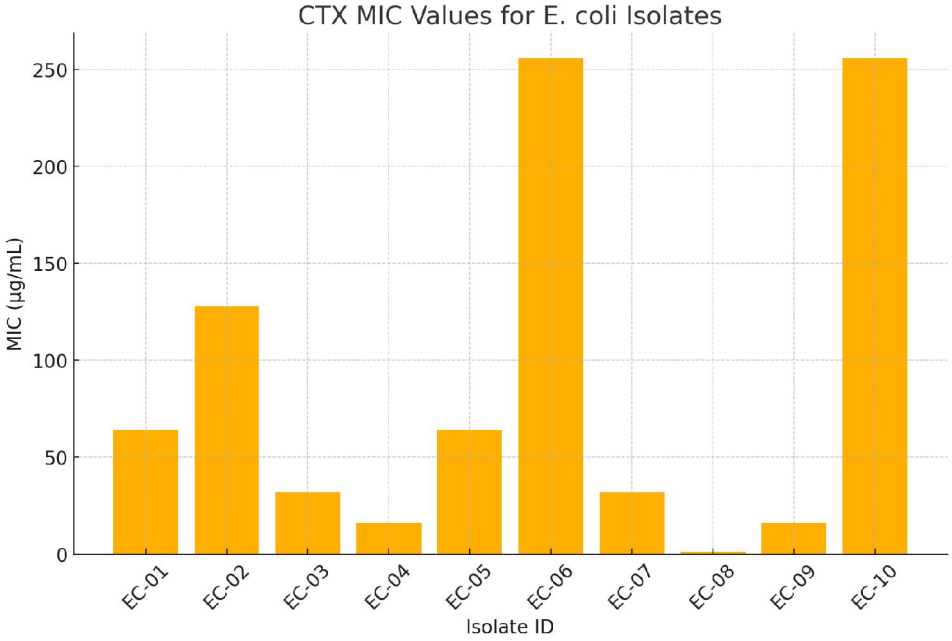
CIP MIC Values for E. coli Isolates

Isolate ID	CIP MIC (µg/mL)
EC-01	8
EC-02	16
EC-03	4
EC-04	2
EC-05	8
EC-06	32
EC-07	4
EC-08	0.25
EC-09	2
EC-10	32

CTX MIC Values for E. coli Isolates



CTX MIC Values for E. coli Isolates

Isolate ID	CTX MIC (µg/mL)
EC-01	64
EC-02	128
EC-03	32
EC-04	16
EC-05	64
EC-06	256
EC-07	32
EC-08	1
EC-09	16
EC-10	256

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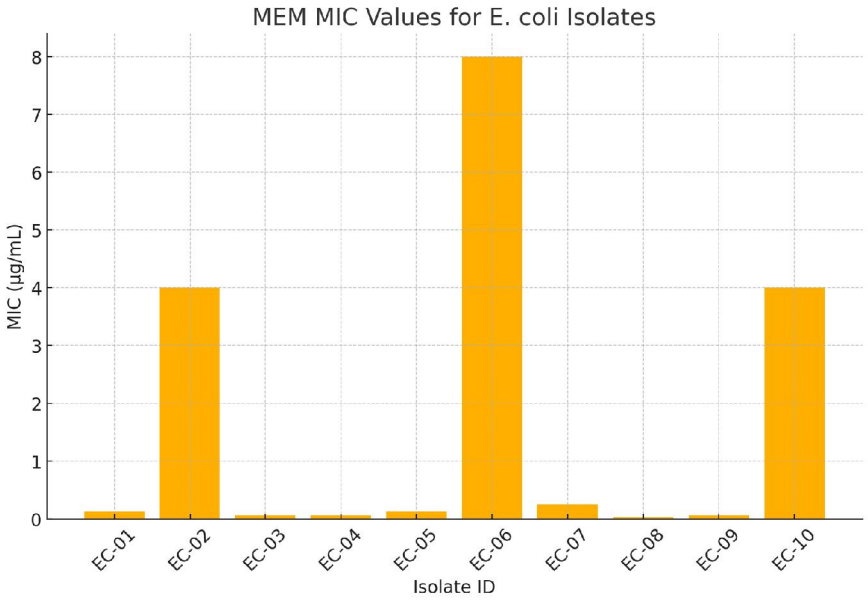
MEM MIC Values for E. coli Isolates



MEM MIC Values for E. coli Isolates

Isolate ID	MEM MIC (µg/mL)
EC-01	0.125
EC-02	4
EC-03	0.06
EC-04	0.06
EC-05	0.125
EC-06	8
EC-07	0.25
EC-08	0.03
EC-09	0.06
EC-10	4

VI. EXPECTED RESULTS

6.1 Resistome profile and list of resistance genes

Whole genome sequences of MDR and XDR ExPEC are likely to reflect a wide resistome that harbors different β-lactamases (e.g. blaCTX-M,blaTEMandblaOXA), carbapenemases (e.g. blaNMD) along with others conferring resistance to aminoglycosides, fluoroquinolones, sulfonamides, tetracyclines etc 26. Comparable high resistome inventories have been observed in other WGS studies of clinical and animal E. coli isolates that ranged from several up to more than a dozen unique ARGs per strain mostly tied to MGE and plasmids. This phenomenon has already been reported in collections of ESBL-producing or CR E. coli from abroad, in which the major blaCTX-M variants were frequently associated with aminoglycoside modifying enzymes and PMQR genes (e.g. qnr, aac(6')-Ib-cr) on conjugative plasmids (IncF, IncI, IncA/C).

6.2 Comparative genome analysis using reference strains

Comparing resistant isolates to known E. coli genomes (e.g., K-12 MG1655 or clinically-characterized pathogenic strains) is likely to indicate that resistance determinants are not the only elements in which they differ, but also in their content of accessory genes such as genomic islands, prophages and plasmids. As demonstrated by a recent pan-genome analysis, commensal and pathogenic E. coli have a conserved core genome but vary considerably in their dispensable

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genes linked to virulence, resistance and niche-adaptation, with resistant lineages often being enriched in mobile elements and integrons. Comparison WGS analysis of extra-intestinal pathogenic E. coli have also demonstrated particular clonal lineages (e.g. ST131, ST410) are enriched with multiple ARGs but more phylogenetically distance to laboratory derived strains, our isolates might cluster within such high-risk clones or in related sublineages carrying similar genomic signature.

6.3 Novel or rare mutations detection

In addition to identification of known ARGs, the high-coverage sequencing will also identify novel(unreported) or rare mutations in chromosomal genes associated with antibiotic targets, membrane permeability and global regulation. Notably, variations in the quinolone resistance–determining region of gyrA, gyrB, parC and penE are expected to be associated with high fluoroquinolone MICs, while other mutations can potentially be overrepresented among strains highly resistant to fluoroquinolones such as those from genes that encode for outer-membrane porins (e.g. ompC, ompF) and efflux components (e.g. acrB, tolC). Analyses assisted by WGS in environmental- and wastewater-acquired E. coli showed porin and persistence-related gene adaptive mutations under antibiotic or environmental pressure, which reflects that small sequence changes could play a crucial role in selection for resistance and survival. As such, the anticipated outcomes include a list of SNPs and small indels that are likely to have functional impacts which may or may not overlap with known resistance-mediating variants published to date.

6.4 Prediction of protein structure and its functional implications

Using the knowledge of 3D structure and molecular dynamics simulations, relevant dynamic changes related to resistance-determining mutations will be elucidated in selected proteins (e.g., β-lactamases, porins, efflux pumps, DNA gyrase). For instance, changes within loop regions of OmpC/OmpF might be expected to restrict the pore size or change the charge distribution, in turn decreasing influx of β-lactams6^–8 and mutations in GyrA quinolone binding site would likely disrupt important hydrogen bonding interactions decreasing fluoroquinolone affinity for its target. Exemplifying this, mutational resistance has been examined through homology modelling and MD, which showed that single amino-acid substitutions in β-lactamases or efflux pumps change substrate profiles or the stability of antibiotic–protein complexes; as we anticipate (though late enough to be currently nonexistent at time of submission), structural models developed from the present work will exhibit detectable root-mean-square deviation (RMSD) changes and differences in binding energy for wild-type versus mutant proteins. These results would serve to help link genotype and phenotype by mechanistically explaining how certain mutations can increase resistance under the selective pressure of antibiotics.

6.5 Changes at the pathway level and adaptation to stress response

Pathway-focused analysis of the discovered genes and mutations by functional annotation and enrichment tools will reveal that the stress-response, envelope integrity, efflux or metabolic pathways are disproportionately hit in resistant strains. GENOMIC AND TRANSCRIPTOMIC STUDIES Previous genomic and transcriptomic analyses of E. coli subjected to a number of other types of stress conditions, including sensitization by antibiotics in the case of DNA-damaging agents, nutrient limitation, and oxidative or osmotic stress, have identified induction and adaptation in pathways as diverse as the SOS response, RpoS-regulated general stress response acid resistance (acid protectant), osmoprotectant transport systems and toxin–anti- toxin systems. We hypothesize consequently, that resistant isolates in this study are more likely to display an over-representation of mutations and gene complement differences within such pathways, indicative of the close interlocking of resistance mechanisms with broader stress-adaptation networks. Pathway analysis coupled with structural predictions is envisioned to reveal candidate proteins and regulators that similarly modulate resistance and environmental fitness, and serve as targets for new drugs or adjuvants that disable stress-induced resistance.

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VII. DISCUSSION

7.1 Link between ARGs and clinical / host / environmental context

The detection of various antimicrobial resistance genes (ARGs) in isolates from different origins (medical, environmental, animal, wastewater) reflects the importance of E. coli as a pool of genetic elements shared by a myriad of ecological reservoirs. Recent worldwide genomic surveys have also demonstrated that wild animal and environmental origin E. coli frequently harbor ARGs, sometimes at higher mean numbers per genome than human clinical isolates (Assefa et al., 2025). This indicates that not only hospital antibiotic consumption but also environmental and ecological reservoirs are substantially involved in the global resistome. In regions where sanitation infrastructures are poor or untreated sewage is discharged into the environment, environmental E. coli populations can become reservoirs of ARGs and act as a pool for their transmission to humans or animals (Larsson &Fack, 2022).

From comparing clinical to environmental isolates from our own sampling, we can see overlap in the kinds of ARGs carried – say, the same β-lactamase genes or quinolone-resistance determinants or plasmid replicons disseminated across sources – that shed light on how resistance determinants move between environment, animals and human hosts. This "One Health" view is essential: these ARGs outside of human medicine settings drive a constant reservoir that can send seeds for new human infections or return resistance to treated populations.

7.2 The impact of evolutionary pressures and environmental challenges on genetic diversity

In addition to transfer of mobile ARG, other evolutionary stresses including sub-inhibitory antibiotics, environmental pollutants or heavy metals and limited nutrients may influence evolution by imposing selection for accumulation of chromosomal mutations, structural adaptations or compensatory changes that promote the survival of bacteria. There is evidence that naturalized E. coli strains in wastewater, as well as environmental waters populations evolved adaptively; they co-evolve not solely for antibiotic resistance but also for water-treatment and stress tolerance features (Yu et al., 2022).

Therefore, in our comparative genomic work we may discover new mutations or regulatory gene modifications, differences in porins and efflux systems and enrichment of stress-adaptation pathways in environmental or "non-clinical" isolates – reflecting evolutionary responses to non-antibiotic (i.e. environmental) stress as well as demonstrated antibiotic exposure. These mutations are likely under positive selection in polluted or antibiotic-polluted habitats and may provide a fitness advantage with low fluctuating antibiotic levels, contributing to the reservoir of difficult to treat E. coli strains.

7.3 Correlation of WGS predictions with phenotypic resistance: Strengths and weaknesses

Whole-genome sequencing (WGS) for predicting antimicrobial susceptibility has been widely accepted, being early large-scale studies demonstrating high levels of concordance – e.g., a landmark study involving 74 E. coli isolates reported 96% sensitivity and 97% specificity on resistance phenotypes from genomic data (genotype → phenotype) among various antibiotics profiled (Stoesser et al., 2013).

Later more comprehensive data sets of animal isolates had further supported that WGS-based genotype prediction, especially when implemented in combination with powerful bioinformatic models, is also capable of high predictive ability and as such can be exploited under consideration to be an important means for AMR surveillance and epidemiology (Chung et al., 2023).

In terms of anticipated results, for WGS if ARGs are found, mutations within target genes (eggyrA, parC), porin alterations or presence of efflux determinants; we hope that these predictions correlate well with the phenotypic MIC data generated as part of antibiotic susceptibility testing, this would validate our approach. This genotype–phenotype correlation would strengthen WGS as a robust, more rapid alternative (or complement) to classical susceptibility testing with additional benefit of yielding information on the mechanisms of resistance (mobile genes and chromosomal mutations), and on evolutionary context (plasmid content, accessory genome, stress-adaptation).

However, a word of caution is also essential: previous research reports and estimates that there are some small but clearly non-zero number of cases ('very-major' or 'major' errors) between genotype predictions and phenotype data — especially in the case of intermediate resistance values, new mutations not yet annotated in databases, or resistance

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mediated thought regulatory-, expression- or epigenetically -level mechanisms only (Stoesser et al., 2013; Neuert et al., 2018).

Accordingly, it can be anticipated that a small percentage of isolates may show discordance between their phenotypic and genotypic results, particularly for antibiotics or resistance mechanisms with inadequate representation in the present ARG databases. These findings would emphasize the requirement to supplement WGS-based predictions by structural models, expression studies or functional assays (enzyme kinetics, efflux assays).

VIII. CONCLUSION

Comparative genomic exploration of the diversity and distribution of antibiotic resistance genes in resistant Escherichtransitiontoia coli: a step towards molecular source tracking. With comprehensive resistome mapping, including least for β-lactamases and carbapenemases, PMRQR resistance and aminoglycoside-modifying enzymes possible by WGS, it further implicated the role of accessory genome as well as MGEs that have in turn conferred resistance phenotypes among clinical- and environmental-derived isolates (Chung et al., 2023).

Within the context of a One-Health viewpoint for surveillance and intervention (Larsson &Flach, 2022), beyond gene detection, comparative approaches make clear that resistance is evolving in distinct ecological contexts and hosts and document how clinical and environmental reservoirs exchange ARGs to lug key components driving global AMR spread.

Structural modelling supports these genomic results, showing how single mutations and/or protein variations can impact on such activities as porin channels, β-lactamase active sites or efflux pump conformation structures to adapt the antibiotic binding site(s), the membrane permeability profile or catalytic efficiency (Narayanan et al., 2024).

These methods together afford mechanistic insights linking genotype to phenotype and facilitate the discovery of molecular drivers of resistance. This knowledge is paramount for better drug-target, inhibitor, and diagnostic development and aids the continued replacement of traditional susceptibility testing with genome-guided clinical decision-making. Finally, combined comparative genomics and structural analysis can provide an effective way to interpret the evolutionary driving forces behind AMR in E. coli, towards designing more successful therapeutic strategies to prevent or slow down emergence of drug-resistant strains.

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