The Study of the Evolution of Antibiotic Resistance in *Escherichia coli*: Genomic Changes and Mechanisms of Resistance

Abstract

The rise of multidrug-resistant *Escherichia coli* poses a significant public health challenge, driven by widespread antibiotic use and complex genomic adaptations. This study investigates the evolution of antibiotic resistance in *E. coli* through comparative genomic analysis of 28 globally sourced strains. Genomes were retrieved from the NCBI database and processed using a standardized bioinformatics pipeline involving sequence trimming, de novo assembly, annotation, and antimicrobial resistance profiling. A total of 252 resistance-related genes were identified, with EF-Tu, MdtABC-TolC, EmrAB-TolC, and AcrAB-TolC among the most prevalent. Mechanisms of resistance included gene mutations, efflux pump overexpression, antibiotic target alteration, and inactivation enzymes. Resistance was most commonly associated with fluoroquinolones, aminoglycosides, and aminocoumarins, with novobiocin resistance being particularly widespread. Phylogenetic analysis revealed significant evolutionary divergence among the isolates, despite a shared ancestor. This divergence correlated with geographic distribution and isolation sources, suggesting adaptation through horizontal gene transfer and environmental pressures. Heatmap visualizations further highlighted resistance gene clustering across antibiotic classes. These findings underscore the urgent need for novel therapeutic strategies targeting conserved elements such as EF-Tu and efflux systems. Inhibitor development against these targets may enhance antibiotic efficacy and curb the spread of resistance. This study contributes to genomic surveillance efforts and offers critical insights into the molecular evolution of resistance in *E. coli*.

Keywords:

Escherichia coli, antibiotic resistance, efflux pumps, EF-Tu, antimicrobial resistance genes, fluoroquinolones, phylogenetics, multidrug resistance , genome analysis

Background

Escherichia coli (*E. coli*), a member of the *Enterobacteriaceae* family, is commonly found in the intestines of humans and animals. It is a leading cause of bacterial infections in both hospital and community settings (Baum & Marre, 2005). Pathogenic *E. coli* is classified into several categories, including Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), *Shigella*/enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), and adherent-invasive *E. coli* (AIEC) (Nasrollahian et al., 2024).

The emergence of multidrug-resistant *E. coli* strains has been accelerated by the misuse and overuse of antibiotics, complicating treatment and increasing mortality rates. The global rise in antibiotic resistance among bacteria is a serious public health concern (Sturmer et al., 2007). Antibiotic resistance poses a significant challenge in *E. coli* strains found in humans, animals, and the environment (Baum & Marre, 2005).

Justification and Purpose of the Study

Escherichia coli serves as a major reservoir for antibiotic resistance genes, leading to treatment failures in both human and veterinary medicine (Poirel et al., 2018). The widespread dissemination of antibiotic-resistant *E. coli* strains presents a growing public health threat (Nasrollahian et al., 2024). The *E. coli* genome consists of approximately 4,000–5,000 genes, with around 3,000 genes shared among various isolates. The remaining genes primarily encode virulence or colonization factors (Poirel et al., 2018).

Horizontal gene transfer, facilitated by mobile genetic elements such as plasmids and transposons, remains the predominant mechanism for acquiring resistance genes (Nasrollahian et al., 2024). Although bacterial antibiotic resistance is a pressing global issue, epidemiological research on this topic varies significantly in approach and is dispersed throughout the literature (Sturmer et al., 2007).

This study aims to investigate the evolution of antibiotic resistance in *E. coli* by identifying the genomic changes and mechanisms that contribute to resistance. The findings will provide insights into resistance mechanisms, inform treatment strategies, and enhance public health interventions.

Aim

To examine the evolution of antibiotic resistance in *Escherichia coli* and identify the genomic changes and mechanisms contributing to resistance.

Objectives

1. To analyze the genomic alterations associated with antibiotic resistance in *E. coli*.
2. To investigate the mechanisms underlying antibiotic resistance in *E. coli*.
3. To evaluate the impact of antibiotic exposure on the evolution of resistance in *E. coli*.
4. To identify potential targets for novel therapeutic approaches and treatment strategies.

Materials and Methods

Data Retrieval

This study engaged in a comparative genomic analysis involving 28 strains of *Escherichia coli*, which were sourced from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). This database affords open-access high-quality genomic data. Each selected strain was supplemented by its corresponding Bio-Project, Bio-Sample, and Sequence Read Archive (SRA) accession numbers, as delineated in Table 1.

The selected strains encompass a diverse array of isolation sources, including clinical, food, animal, and environmental origins. Moreover, these strains have been procured from various geographical locales including North America, South America, Asia, Europe, and Africa. This strategic selection was intended to capture the global genomic variability of E. coli, thereby enhancing the comprehensiveness of the resultant analyses.

All genome sequences were generated utilizing Illumina sequencing platforms, which provided reliable, high-throughput, short-read data appropriate for bacterial genome assembly, annotation and comparative analyses. The resultant dataset was fundamental in facilitating investigations related to bacterial evolution, pathogenicity and patterns of antimicrobial resistance, thus contributing to broader initiatives in genomic surveillance and epidemiological research. A detailed enumeration of strains and their associated metadata is presented in Table 1.

Table 1: A full genome assembly table for 28 *Escherichia coli* strains

Bio project	Bio sample	SRA	Sample ID	Size (MB)	Region	Isolation source	Submitter	Year	Sequencing platform	Assembly
PRJNA230969	SAMN04913923	SRR3951549	MODI-EC4326	845	USA	Environment	FDA Center for Food Safety and Applied Nutrition (CFSAN)	Oct 6, 2017	Illumina	GCA_002475705.1
PRJNA606985	SAMN14118537	SRR11094155	SCHI 0016.S.230	518.4	Australia: Sunshine Coast	urine	National Center for Biotechnology Information	Apr 28, 2020	Illumina	GCA_012919845.1
PRJEB72521	SAMEA116822488	ERR13934899	uvzsr-PT_24_00030-A02-AR_24_00002707	388.3	Slovakia	secretion	EBI	Nov 8, 2024	Illumina	GCA_964338715.1

PRJEB72521	SAMEA11707098	ERR13947894	uvzsr-PT_24_00031-A07-AR_24_00002423	572.9	Slovakia	rectal swab	EBI	Nov 13, 2024	Illumina	GCA_964339345.1
PRJNA293225	SAMN068 48977	SRR5649668	NC_C18-16	311.4	Uganda	Cattle	US Food and Drug Administration	Aug 31, 2020	Illumina	GCA_014347275
PRJNA230968	SAMN02628668	SRR1269355	AZ-TG73659	407.2	USA:MI	thin sliced breast	FDA/CFSAN	Apr 24, 2020	Illumina	GCA_012833455.1
PRJNA357722	SAMN06680929	SRR5470825	PSU-0064	320.7	USA: OH	lettuce	FDA	Apr 17, 2020	Illumina	GCA_012476595.1
PRJNA516477	SAMN10821522	SRR8541398	RS218	498.1	Canada	urine	National Center for Biotechnology Information	Jul 30, 2020	Illumina	GCA_013969365.1
PRJNA523709	SAMN10986378	SRR8610355	121 HS-D	1.1Gb	Tanzania		National Center for Biotechnology Information	Apr 15, 2020	Illumina	GCA_012298495.1
PRJNA523709	SAMN10986369	SRR8610352	131 HS-A	1.4Gb	Tanzania		National Center for Biotechnology Information	Apr 15, 2020	Illumina	GCA_012298585.1
PRJEB59977	SAMEA112957168	ERR11268661	DKB0435	406.5	Uganda	pig	National Center for Biotechnology Information	Oct 3, 2023	Illumina	GCA_032302515.1
PRJNA230969	SAMN10253046	SRR8072059	FE95610	323.5	Burkina Faso	beef meat	FDA/CFSAN	Apr 6, 2020	Illumina	GCA_012078575.1
PRJNA218110	SAMN07169275	SRR5605996	00-3003	412.7	USA	stool	CDC	May 13, 2020	Illumina	GCA_013076505.1
PRJNA357722	SAMN06680897	SRR5470914	10.0554	510.2	USA	Food	FDA	Apr 17, 2020	Illumina	GCA_012476375.1
PRJNA503851	SAMN40946983	SRR28645744	ECOL-23-VL-ON-PE-	432	Canada: Prince Edward Isl	ear	Vet-LIRN-CVM-FDA	Apr 15, 2024	Illumina	GCA_038110005.1
PRJNA578368	SAMN13058769	SRR10310383	108-19	457.9	Brazil: Londrina	urine	National Center for Biotechnology Information	Feb 26, 2021	Illumina	GCA_016991595.1

PRJNA934699	SAMN33277588	SRR23448805	BT2	502.4	China:Guangzhou	soil	National Center for Biotechnology Information	Feb 17, 2023	Illumina	GCA_028672105.1
PRJNA944266	SAMN33746135	SRR23851410	MFDS1001886	469	South Korea: Busan	food	National Center for Biotechnology Information	Nov 18, 2024	Illumina	GCA_045006045.1
PRJNA230969	SAMN03952738	SRR2174046	MOD1-EC1236	487	USA	food	FDA/CFSAN	Feb 21, 2025	Illumina	GCA_012803915.2
PRJNA218110	SAMN46534118	SRR32216821	PNUSAE205182	355	USA		CDC	Feb 18, 2025	Illumina	GCA_047843005.1
PRJNA230969	SAMN39473807	SRR27594984	1382-3-stec	356	USA:CA	"feces, wildlife"	FDA/CFSAN	Jan 18, 2024	Illumina	GCA_035821635.1
PRJNA292667	SAMN19796345	SRR14871403	FSIS12140060	346.7	USA:MD	"Product-Raw-Intact-Siluriformes\, Ictaluridae (Catfish)"	FDA	Jun 22, 2021	Illumina	GCA_018998645.1
PRJNA1078256	SAMN39992794	SRR28012662	KTa009	312.2	Japan	river water	National Center for Biotechnology Information	Oct 11, 2024	Illumina	GCA_043017345.1
PRJNA268206	SAMN35158809	SRR24640677	FSIS12322051	449.6	USA:OH	comminuted beef	National Center for Biotechnology Information	Nov 10, 2023	Illumina	GCA_033510075.1
PRJNA218110	SAMN46756473	SRR32299115	PNUSAE205432	395.9	USA		CDC	Feb 10, 2025	Illumina	GCA_047607815.1
PRJNA218110	SAMN12598049	SRR9993950	PNUSAE030010	312.8	USA		CDC	Feb 22, 2025	Illumina	GCA_011886765.2
PRJNA418674	SAMN03083281	SRR5242761	WCHEC005008	481.2	China: Chengdu	abdominal drainage	West China Hospital,	Feb 19, 2019	Illumina	GCA_001014795.2
PRJNA655603	SAMN15746609	SRR12398593	STEC	636.9	Australia: Queensland	human	National Center for Biotechnology	Jun 24, 2021	Illumina	GCA_019023825.1

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Genome Assembly and Quality Assessment

Raw sequence reads underwent processing via Trim Galore v0.6.5dev to eradicate adapter sequences and low-quality bases. The resultant high-quality reads were subsequently assembled de novo using Unicycler v0.4.8. Furthermore, the assemblies were polished through the application of Pilon v1.23 to rectify any misassembles and enhance base accuracy. A detailed enumeration of strains and their associated metadata is presented in Table 2.

The quality of the assemblies was assessed using QUAST v5.2.0, which provided critical metrics such as N50, genome length, GC content and the number of contigs. Confirmatory read mapping and indexing were achieved using samtools v1.13 to validate assembly completeness. Visualization of the assembly graphs was conducted using Bandage. The finalized assemblies were subsequently submitted to the BV-BRC Genome Assembly and Annotation Pipeline v3.46.3 for standard annotation and feature extraction.

Table 2: A full genome annotation table for 28 *Escherichia coli* strains

SAMPLE	PLASMID	GENES LENGTH (BP)	GC CONTENTS	ORF	ANNOTATED GENE	HYPOTHETICAL GENES	GENES WITH PFAM	RNA GENES	CRISPR	CONTIGS	CONTIGS N50
MODI - EC4326	0	5,319,590 bp	50.28	5,518	4,873	645	5,387	94	4	243	146,357
SCHI 0016.S.230	0	4,727,083 bp	50.75	4,587	4,180	407	4,501	71	0	45	341,812
uvzsr-PT_24_00030-A02-AR_24_00002707	0	5,044,961 bp	50.55	5,033	4,442	591	4,914	84	6	180	100,804
uzsr-PT_24_00031-A07-AR_24_00002423	0	5,029,715 b	50.52	5,052	4,498	554	4,919	81	0	124	186,659
NC_C18-16	0	4,357,241 bp	50.94	4,305	3,928	377	4,235	81	40	64	209,348
AZ-TG73659	0	4,975,989 bp	50.53	4,849	4,328	521	4,751	86	53	64	209,242

PSU-0064	0	4,964,948 b	50.48	5,177	4,620	557	5,067	93	0	257	95389
RS218	0	4,817,112	50.53	4,766	4,279	487	4,653	75	0	97	215,957
121 HS-D	0	4,379,975	50.74	4,359	4,011	348	4,280	79	4	107	125,490
131 HS-A	0	4,983,314	50.65	4,988	4,475	513	4,877	83	0	137	247,636
DKB0435	0	4,947,849	50.67	4,940	4,477	463	4,837	81	0	95	210,287
FE95610	0	4,785,388	50.88	4,769	4,264	505	4,660	85	0	123	182,079
00-3003	0	4,818,165	50.70	5,111	4,495	616	4,975	83	0	301	51,792

SAMPLE	PLASMID	GENES LENGTH (BP)	GC CONTENTS	ORF	ANNOTATED GENE	HYPOTHETICAL GENES	GENES WITH PFAM	RNA GENES	CRISPR	CONTIGS	CONTIGS N50
00-3003	0	4,818,165	50.70	5,111	4,495	616	4,975	83	0	301	51,792
10.0554	0	4,634,767	50.58	4,917	4,442	475	4,798	100	0	280	92,251
ECOL-23-VL-ON-PE-	0	4,440,512	50.88	4,456	4,084	372	4,378	92	0	105	231,355
108-19	0	4304509	50.90	4,286	3,954	332	4,214	93	13	117	85,765
BT2	0	4,355,828	50.92	4,329	3,996	333	4,253	83	11	115	142,810
MFDS1001886	0	4,579,369	50.59	4,625	4,134	491	4,522	90	26	164	84,491
MOD1-EC1236	0	4,677,592	50.56	4,944	4,440	504	4,834	98	0	275	137,686
PNUSAE205182	0	4,772,988	50.69	5,111	4,488	623	4,986	100	0	280	89,761
1382-3-stec	0	5,451,454	50.30	5,851	5,156	695	5,697	100	19	358	107,312
FSIS12140060	0	4,527,146	50.93	4,383	4,100	283	4,331	81	7	70	138,183
PNUSAE030010	0	5,306,801	50.25	5,506	4,869	637	5,388	97	4	239	187,937
PNUSAE205432	0	4,465,952	50.48	4,839	4,341	498	4,701	81	0	375	31,293
FSIS12322051	0	4,993,852	50.46	5,193	4,619	574	5,057	101	7	319	104,128
KTa009	0	5,110,674	50.59	5,158	4,438	720	5,050	79	16	140	125,336
WCHEC005008	0	4,970,882	50.57	4,986	4,464	522	4,873	77	20	162	106,192
STEC	0	5,148,976	50.23	5,334	4,717	617	5,217	92	4	242	148,119

Genome Annotation and Functional Characterization

The annotated genomes underwent processing through the BV-BRC pipeline, which identified coding sequences (CDSs), RNA genes, CRISPR elements and functional protein domains, including Pfam and TIGRFAMs. This rigorous analysis facilitated the identification of potential virulence factors and hypothetical proteins. Summary statistics concerning gene content, GC content, and hypothetical proteins are expounded in Table 2.

Antimicrobial Resistance Profiling

The identification of antimicrobial resistance (AMR) genes for each strain was accomplished through the utilization of integrated tools within the BV-BRC annotation suite, referencing established resistance gene databases. Resistance determinants were systematically classified according to their associated drug classes and individual antibiotics.

Data Structuring and Grouping for Comparative Analysis

Grouping and Analysis of AMR Data

The identified antimicrobial resistance genes were subjected to systematic analysis and classification based on the following dimensions:

- **Strain vs Gene**: Identification of specific resistance genes present in each strain of E. coli.
- **Strain vs Antibiotic Class**: Classification of resistance according to the associated drug class (e.g., beta-lactams, aminoglycosides) pertinent to the detected genes.
- **Strain vs Antibiotics**: Identification of specific antibiotics to which each strain exhibits genetic resistance.
- **Strain vs Classification**: Grouping of strains based on their resistance status (e.g., multidrug-resistant, extensively drug-resistant).
- **Gene vs Antibiotic Class**: Mapping of resistance genes to the drug classes for which they confer resistance.
- **Gene vs Classification**: Association of each gene with its corresponding resistance classification.
- **Antibiotic Class vs Classification**: Assessment of the relationship between antibiotic classes and their respective resistance classifications.

These structured comparisons facilitated a comprehensive understanding of the distribution of resistance traits across strains and drug categories.

Heatmap Generation and Visualization

Data matrices derived from the AMR groupings were employed in the construction of heatmaps using RStudio v4.3.0, utilizing the pheatmap package. These heatmaps effectively visualize the presence and intensity of resistance genes across strains, categorized by antibiotic class and classification levels. This methodological approach aided in the identification of trends and potential hotspots of resistance.

Phylogenetic Analysis

A phylogenetic analysis was conducted to show the evolutionary relationships among the 28 strains of *Escherichia coli*, predicated on whole-genome data. Two complementary tools were employed for this analysis:

1. Phylogenetic Tree Construction Utilizing BV-BRC

The overarching phylogenetic tree was constructed using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). The analysis incorporated whole-genome alignments to generate a rooted phylogenetic tree, complete with bootstrap values derived from replications and branch lengths determined automatically by the BV-BRC pipeline. This methodological framework provided a statistically robust basis for evaluating the evolutionary relationships among the strain's profiles and genetic lineages.

2. Tree Visualization using MEGA

The tree file obtained from BV-BRC was imported into MEGA (Molecular Evolutionary Genetics Analysis) software for enhanced visualization and annotation. MEGA allowed for clearer formatting of strain labels and more precise representation of branch lengths and bootstrap values. No additional tree-building computation was done in MEGA; it was used purely for presentation purposes.

The final phylogenetic tree highlighted clusters of closely related strains, offering insight into possible evolutionary paths and relationships between antimicrobial resistance profiles and genetic lineages.

RESULT

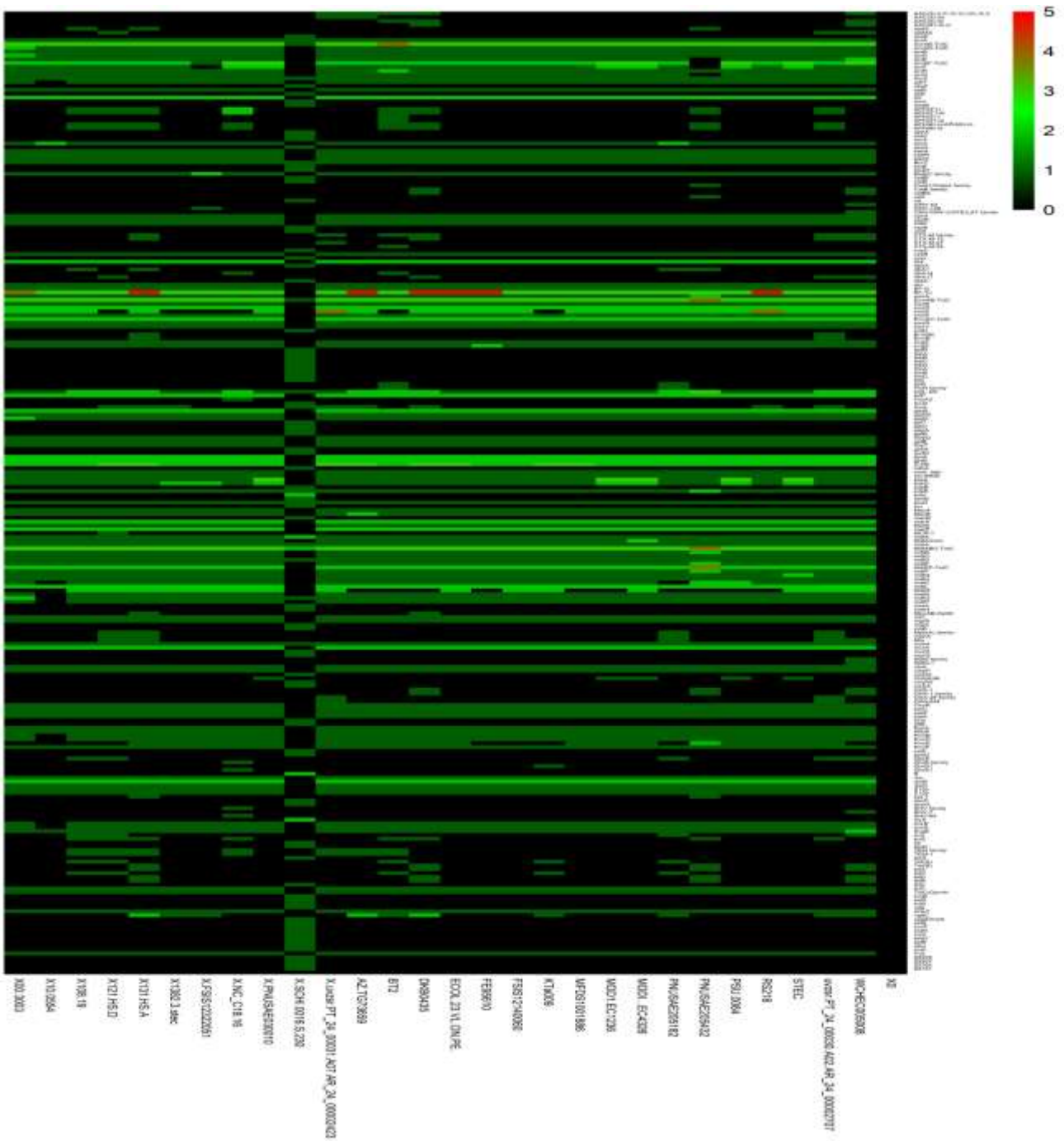

Fig 1. Heatmap of Strains vs genes

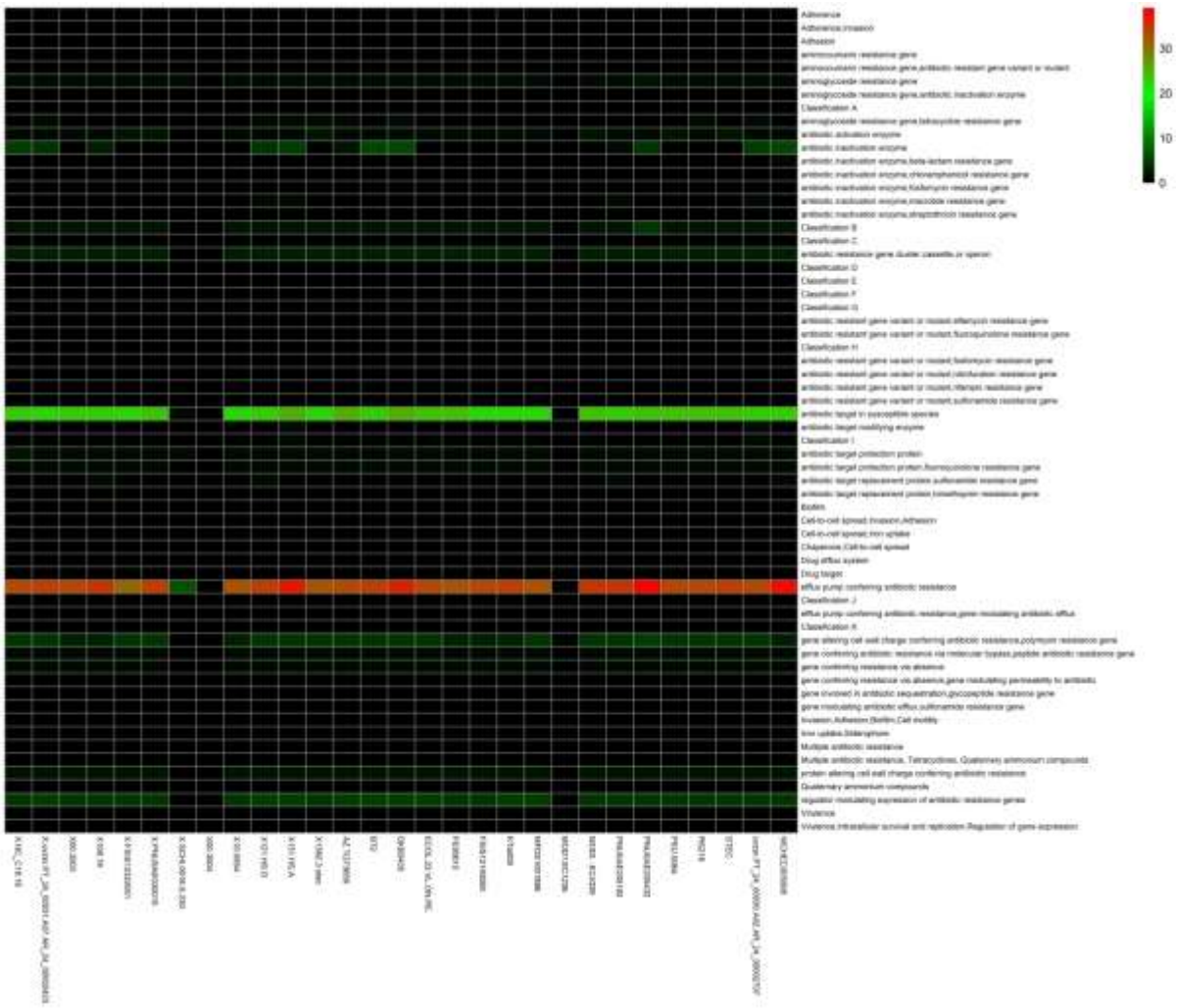

Fig 2. Heatmap of Strain vs Classification

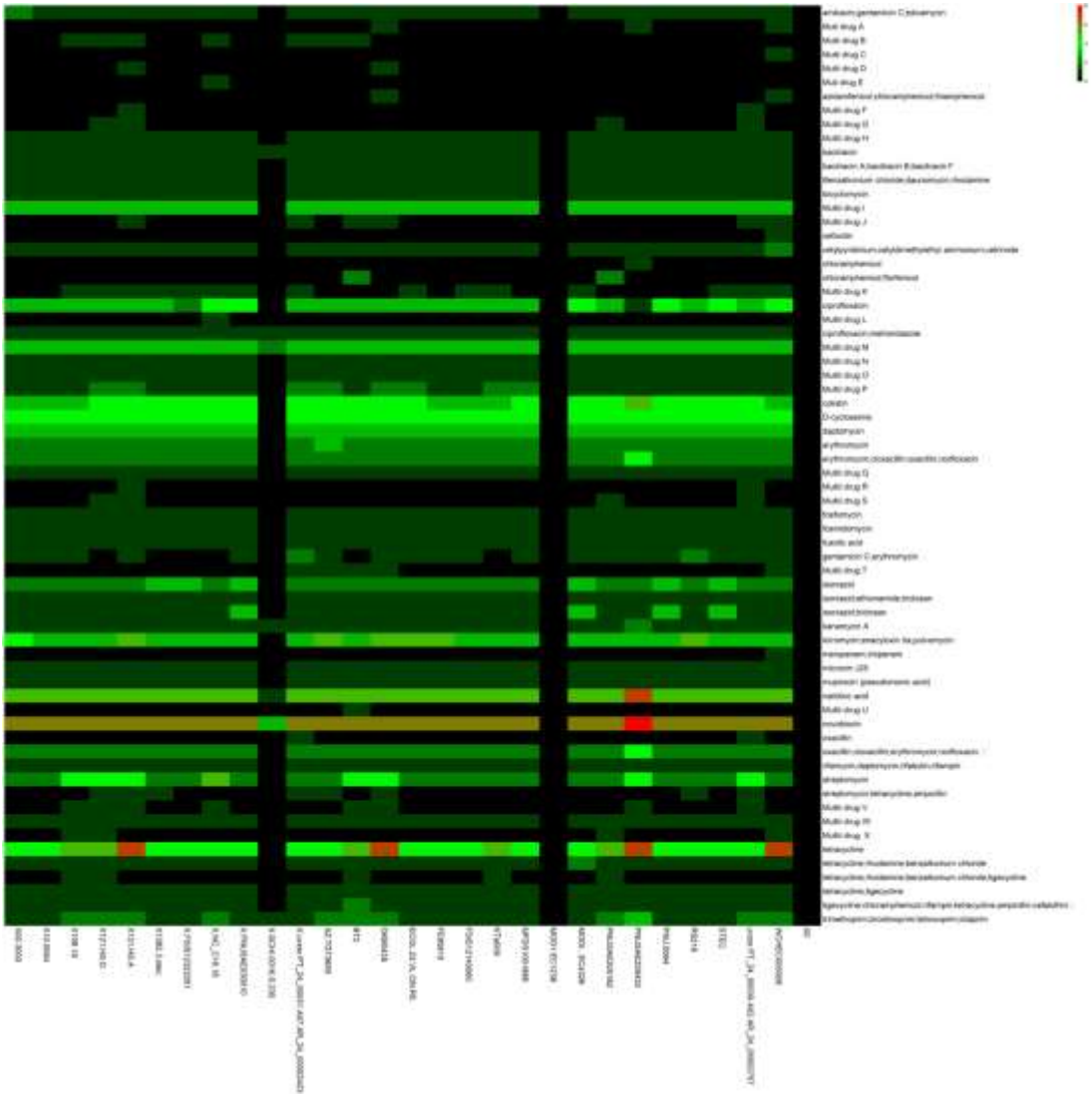

Fig. 3 Heatmap of Strains vs Antibiotic

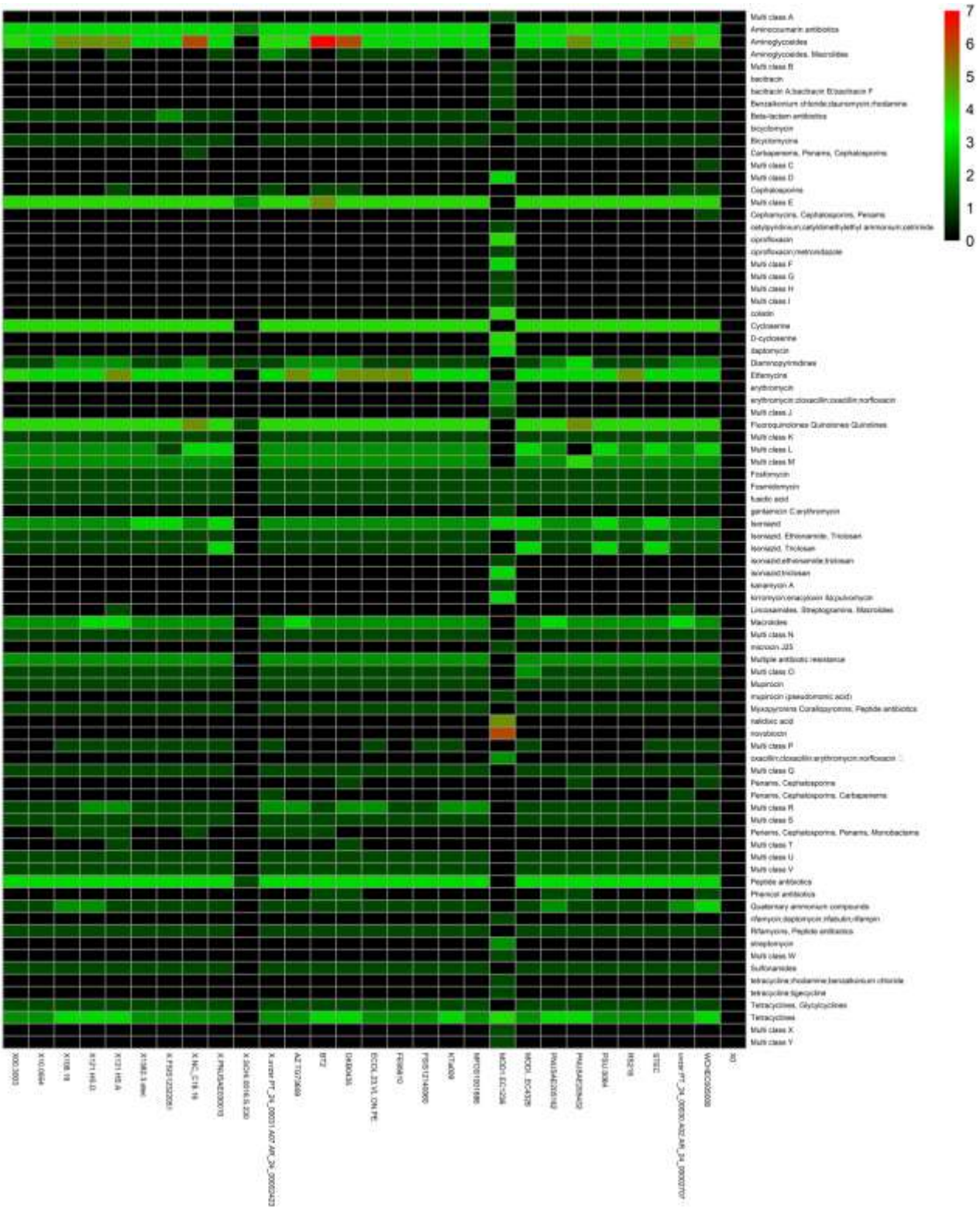

Fig 4. Heatmap of Strains vs Antibiotic class

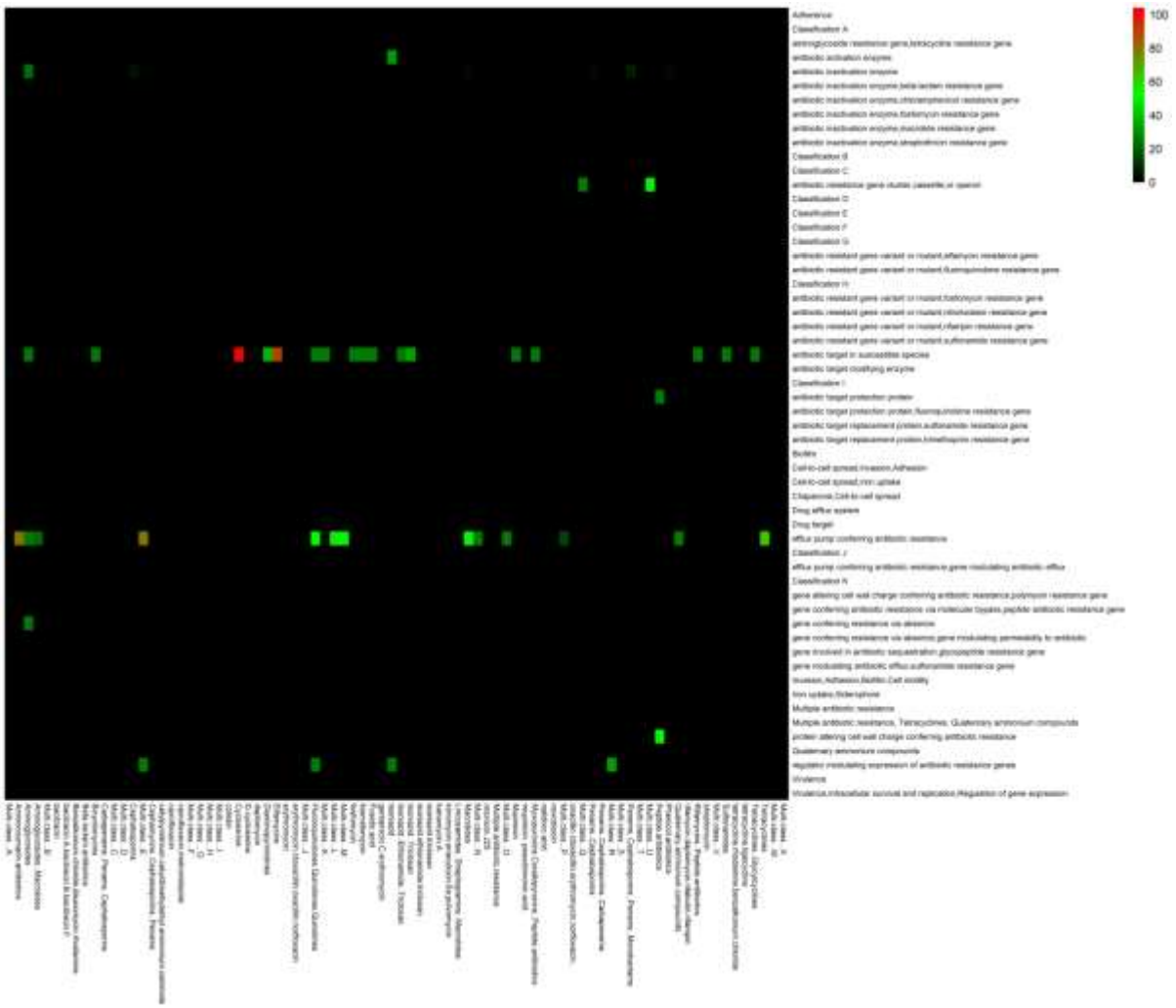

Fig. 5. Heatmap of Antibiotic Class vs Classsification

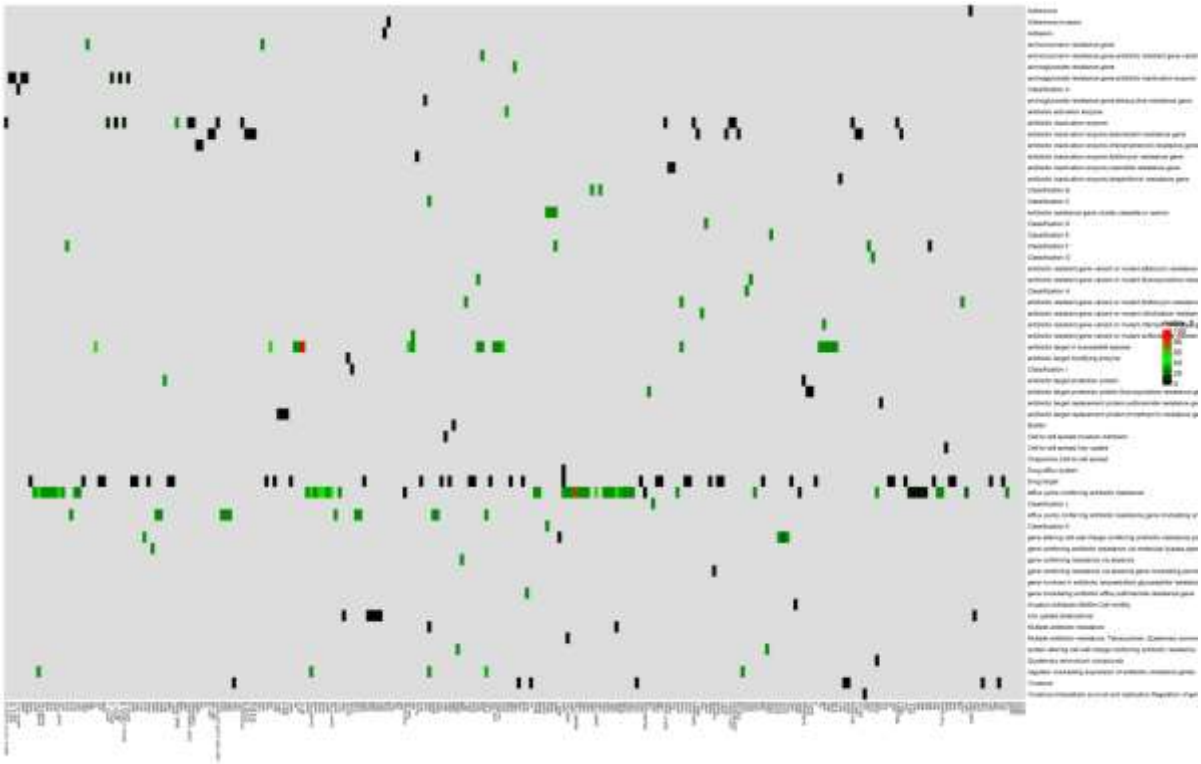

Fig. 6. Heatmap of Gene vs Classification

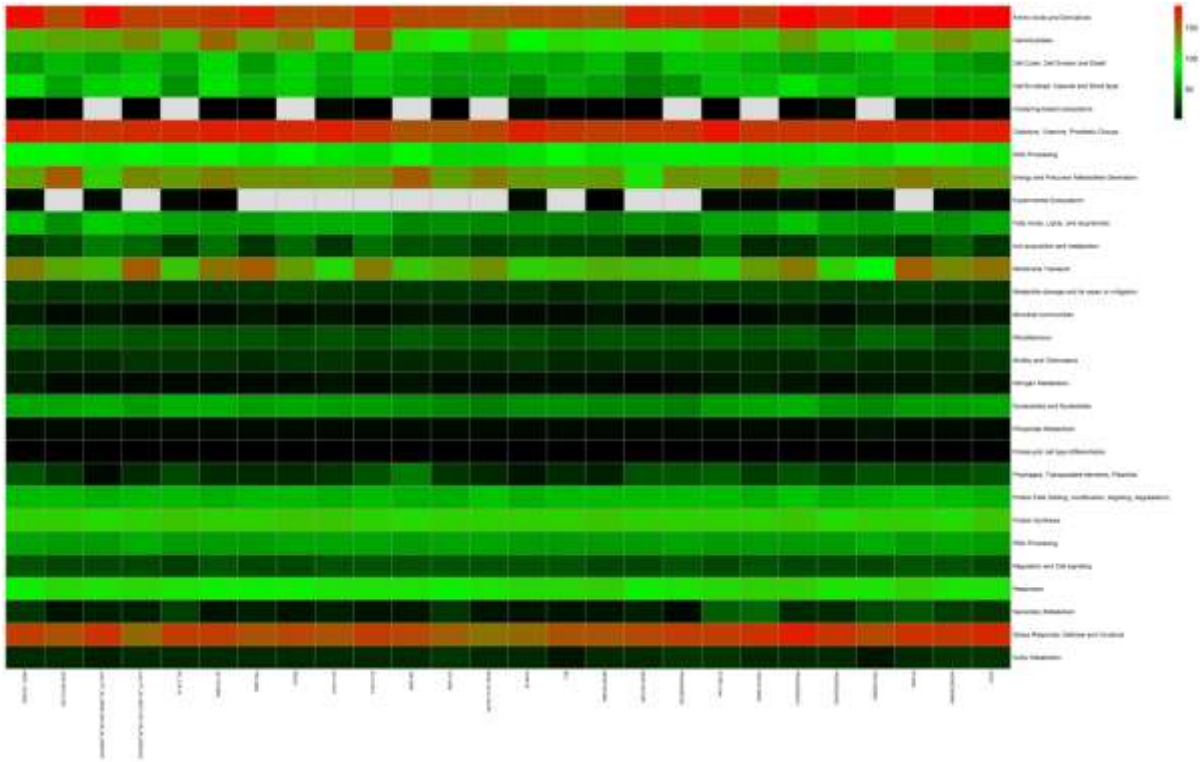

Fig. 7 E. coli Subsystems

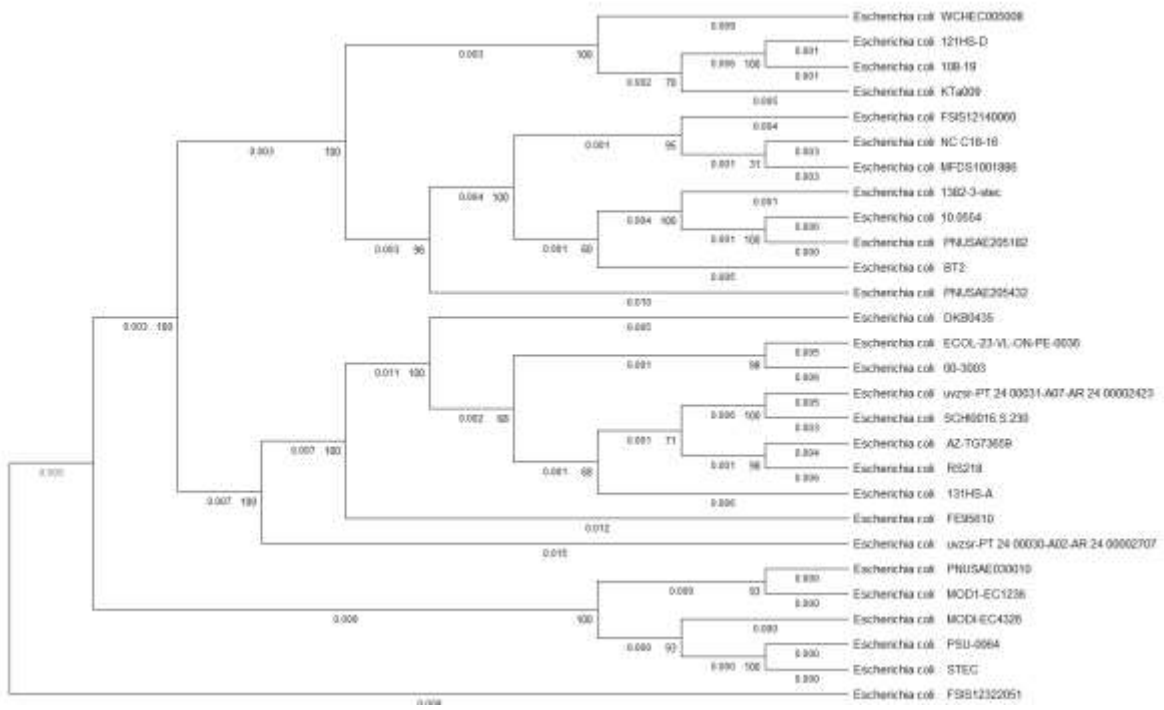

Fig.8 Phylogenetic tree of E. coli strains

DISCUSSION

This study revealed that *Escherichia coli* employs multiple defence and protection mechanisms against antimicrobial agents. These include gene mutations, upregulation of resistance genes, adaptive resistance, and the acquisition of specific resistance genes.

A total of 252 genes were identified across 28 *E. coli* isolates. These genes encode proteins responsible for various cellular functions that support E. coli's survival, growth, and environmental interactions. Notably, most of the identified genes were associated with antimicrobial resistance.

The study also demonstrated that many of these genes have undergone mutations via insertion or deletion events—entailing the addition or removal of nucleotides—as well as duplication and recombination. For instance, the AAC(6')-Ib-cr gene, a variant of AAC(6')-Ib, modifies aminoglycosides and reduces their effectiveness. It also possesses the unique ability to acetylate certain fluoroquinolones, rendering them less effective. Similarly, the aadA5 gene, a variant of the aadA gene, encodes an aminoglycoside adenylyltransferase enzyme that modifies antibiotics like streptomycin and spectinomycin through adenylylation. These genetic changes have altered antibiotic target sites and efflux pumps, conferring resistance and increasing the virulence of *E. coli* strains. These more virulent strains can cause a range of diseases, from mild to life-threatening.

Among all the genes isolated, EF-Tu was the most predominant, followed by MdtABC-TolC, EmrAB-TolC, and AcrAB-TolC, as shown in Figure 1. The EF-Tu gene, which encodes Elongation Factor Thermo-unstable, is essential for protein synthesis during the elongation phase (Prezioso et al., 2017). EF-Tu plays a crucial role in protein translation, which impacts growth, development, and cellular responses to stress (Hughes, 2013). The study indicated that antibiotics exert their action on EF-Tu by targeting it in susceptible species, as illustrated in Figure 6. Mutations in this gene can alter the EF-Tu protein structure and function, contributing to antibiotic resistance.

The MdtABC-TolC, EmrAB-TolC, and AcrAB-TolC genes encode multidrug efflux pumps that export toxic substances, including antibiotics, out of the bacterial cell. This mechanism reduces the intracellular concentration of antibiotics and promotes multidrug resistance (Sun et al., 2014).

Multiple resistance mechanisms were identified, including antibiotic target-modifying enzymes, target protection and replacement proteins, antibiotic inactivation enzymes, gene variants, gene clusters or operons, efflux pumps, cell wall-altering genes, permeability-modulating genes, genes involved in antibiotic sequestration, and biofilm formation. Other identified mechanisms included drug targets and efflux systems, regulatory elements controlling resistance gene expression, and virulence-associated traits such as adhesion, invasion, motility, iron uptake, and intracellular survival.

Resistance genes were classified according to antibiotic classes, including those for fluoroquinolones, tetracyclines, β-lactams, sulfonamides, chloramphenicol, fosfomycin, aminocoumarins, aminoglycosides, trimethoprim, nitrofurantoin, rifampin, lincosamides, macrolides, streptogramins, elfamycins, streptothricins, polymyxins, and glycopeptides.

Efflux pump-mediated resistance was the dominant mechanism among the *E. coli* strains, as depicted in Figure 2. Efflux genes identified include MdtABC-TolC, EmrAB-TolC, AcrAB-TolC, MacA, MacB, mdfA, MdfA/Cmr, mdtA through mdtP, MexAB-OprM, msbA, patA, QacE, SugE, Tet(A–D), tolC, TolC/OpmH, vgaC, and YojI (Figure 6). Overexpression of these genes may result from mutations in regulatory elements or insertion of IS elements upstream, which act as strong promoters (Nasrollahian et al., 2024). Multidrug efflux systems are known to export a wide array of substrates, including various antibiotics (Li and Nikaido, 2009; Grossman, 2016), and their overexpression has been strongly linked to clinical antibiotic resistance (Sun et al., 2014).

The study found high resistance levels in *E. coli* strains to several antibiotics, with novobiocin showing the highest resistance (Figure 3). Novobiocin, an aminocoumarin

antibiotic that inhibits DNA synthesis, is no longer commonly used due to its toxicity and limited effectiveness (Dahal, 2023). Resistance to novobiocin was linked to efflux pump activity (Figure 5).

High resistance levels were also noted for fluoroquinolones, quinolones, Multi Class E antibiotics, aminoglycosides, and cycloserine (Figure 4). The most common mechanism of action by these antibiotics was targeting susceptible species, a mechanism that was particularly resisted by mutations in the EF-Tu gene (Figure 5).

Fluoroquinolones, essential for treating a wide range of infections in humans and animals, differ from quinolones by the presence of a fluorine atom at the C-6 position (Block & Blanchard, 2025; DeepakRajput, 2023). Resistance in *E. coli* strains spanned all generations of fluoroquinolones, from nalidixic acid to moxifloxacin, and was linked to efflux pumps, regulatory mutations, and EF-Tu gene alterations.

Aminoglycosides target the 30S ribosomal subunit, specifically binding to the A-site of 16S rRNA, thereby disrupting protein synthesis (Poirel et al., 2018; Block & Blanchard, 2025). Resistance mechanisms identified included gene absence, antibiotic inactivation enzymes, and EF-Tu gene mutations that inhibit the drugs' action (Figure 5).

Cycloserine, used in treating urinary tract infections, tuberculosis, conjunctivitis, and skin infections, inhibits cell wall synthesis (Sharma, 2025). Its action, targeting susceptible species, was also thwarted by EF-Tu gene mutations (Figure 5).

Multi Class E includes cephalosporins, penams, tetracyclines, glycyclines, phenicol antibiotics, rifamycins, fluoroquinolones, quinolones, and triclosan. Resistance mechanisms against these included efflux pumps and regulators modulating resistance gene expression (Figure 5).

Investigation of the strains of *E. coli* revealed 30 subsystems within the genome that functions together to carry out specific biological processes of the organism. These functional interactions coordinate the activities of the organism (Shimizu, 2013).

Among the 30 subsystems, Amino acids and derivatives were the most prevalent, followed by cofactors, vitamins, and prosthetic groups, stress response, defense and virulence, energy and precursor metabolites generation, membrane transport, carbohydrate, protein synthesis, respiration and DNA processing as shown in Figure 7. These subsystems contribute significantly to the ability of *E. coli* to sense, integrate, and respond to a variety of stresses for survival (Shimizu, 2013).

THE PHYLOGENETIC TREE

The constructed phylogenetic tree (Figure 8) demonstrated genetic relatedness and divergence among the *E. coli* isolates, all descending from a common ancestor, *Escherichia coli* FSIS12322051, originally from the USA. Despite their common origin, the isolates have diversified significantly.

For example, isolates such as *E. coli* AZ-TG73659 and FSIS12140060 from the USA exhibited considerable divergence, likely due to differing habitats or sources of isolation, indicating distinct pathogenic profiles.

Similarly, E. coli MFDS1001886 (South Korea) and MOD1-EC1236 (USA) diverged evolutionarily despite being foodborne. Likewise, *E. coli* RS218 (Canada) and 108-19 (Brazil), both isolated from urine, showed high divergence—possibly due to geographic separation and environmental pressures promoting unique traits.

Conversely, isolates such as *E. coli* 10.0554 and PNUSAE205182 from the USA displayed high genetic similarity, suggesting a common transmisssion route within the region.

Interestingly, *E. coli* 121 HS-D (Tanzania) and 108-19 (Brazil) exhibited close genetic relatedness despite originating from different continents, suggesting possible cross-border transmission.

Outliers like *E. coli* STEC (Australia) and 10.0554 (USA), located on the tree's terminal branches, showed the greatest divergence, raising concerns about the emergence of novel, potentially more virulent lineages.

In summary, the 28 *E. coli* isolates analyzed in this study are evolutionarily derived from a common ancestor. Their genetic diversity appears driven by adaptation and mutations through insertions and deletions.

CONCLUSION AND RECOMMENDATION

The genetic structure of *Escherichia coli* forms the foundation of its antimicrobial resistance. The evolutionary divergence observed among strains reflects the organism's response to environmental pressures. These pressures have refined E. coli's genome to optimize replication and survival, often through the development of resistant variants via mutations.

To counteract this challenge, new therapeutic strategies should specifically target the EF-Tu gene and multidrug efflux pumps such as MdtABC-TolC, EmrAB-TolC, and AcrAB-TolC. Developing inhibitors against these targets could enhance antibiotic effectiveness and reduce resistance. Inhibiting EF-Tu activity, in particular, may impair bacterial protein synthesis, offering a potential pathway to reduce *E. coli* viability and pathogenicity.

Genomic data such as the findings presented here will aid in the interpretation of data from future outbreaks, management and treatment.

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Appendix

DEFINITION OF TERMS AND ABBREVIATIONS

Classification A - aminoglycoside resistance gene, antibiotic inactivation enzyme, fluoroquinolone resistance gene

Classification B - antibiotic resistance gene cluster, cassette, or operon, efflux pump conferring antibiotic resistance

Classification C - antibiotic resistance gene cluster, cassette, or operon, efflux pump conferring antibiotic resistance, gene modulating antibiotic efflux

Classification D - antibiotic resistant gene variant or mutant, beta-lactam resistance gene,gene modulating permeability to antibiotic

Classification E - antibiotic resistant gene variant or mutant,efflux pump conferring antibiotic resistance,gene altering cell wall charge conferring antibiotic resistance,gene modulating antibiotic efflux,polymyxin resistance gene

Classification F - antibiotic resistant gene variant or mutant,efflux pump conferring antibiotic resistance,gene modulating antibiotic efflux

Classification G - antibiotic resistant gene variant or mutant,efflux pump conferring antibiotic resistance,gene modulating antibiotic efflux,gene modulating permeability to antibiotic

Classification H - antibiotic resistant gene variant or mutant,fluoroquinolone resistance gene,gene involved in self resistance to antibiotic

Classification I - antibiotic target modifying enzyme,lincosamide resistance gene,macrolide resistance gene,streptogramin resistance gene

Classification J - efflux pump conferring antibiotic resistance,gene altering cell wall charge conferring antibiotic resistance,gene conferring resistance via absence,gene modulating antibiotic efflux,polymyxin resistance gene

Classification K - efflux pump conferring antibiotic resistance,gene modulating antibiotic efflux,gene modulating permeability to antibiotic

Multi drug A - amoxicillin, cephalothin, piperacillin, piperacillin/tazobactam, oxacillin

Multi drug B - amoxicillin/clavulanic acid, amoxicillin, ampicillin, cephalothin

Multi drug C - amoxicillin/clavulanic acid, cefoxitin, ertapenem, imipenem, meropenem, piperacillin/tazobactam, ticarcillin

Multi drug D - ampicillin, amoxicillin/clavulanic acid, azithromycin;aztreonam, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, colistin, erythromycin, meropenem, nalidixic acid, novobiocin, panipenem, tetracycline;ticarcillin, trimethoprim, trimethoprim/sulfamethoxazole, sulfamethoxazole

Multi drug E - ampicillin, cefazolin, ceftriaxone, cephalothin

Multi drug F - azithromycin, clarithromycin, clindamycin, dalfopristin, dirithromycin, erythromycin, griseoviridin, lincomycin;madumycin II, oleandomycin;ostreogrycin B3, patricin A, patricin B, pristinamycin IA, pristinamycin IB, pristinamycin IIA, quinupristin, roxithromycin, spiramycin, telithromycin, tylosin, vernamycin B-gamma, vernamycin C, virginiamycin S2

Multi drug G - azithromycin, clarithromycin, dirithromycin, erythromycin, oleandomycin, roxithromycin, telithromycin

Multi drug H - azithromycin, gentamicin, nitrofurantoin, oxacillin, spiramycin

Multi drug I - cefalothin, chloramphenicol, tigecycline, ampicillin, tetracycline, rifampin

Multi drug J - cefotaxime, ceftriaxone, cephalothin, cephalothin

Multi drug K - chloramphenicol, norfloxacin, puromycin, lincomycin, acriflavin

Multi drug L - ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, nalidixic acid, norfloxacin, sparfloxacin

Multi drug M - ciprofloxacin, tigecycline, chloramphenicol, rifampin, tetracycline, ampicillin, cefalothin

Multi drug N - clofazimine, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, nalidixic acid;ofloxacin, sparfloxacin, trovafloxacin

Multi drug O - clofazimine, gatifloxacin, ciprofloxacin, levofloxacin, moxifloxacin, nalidixic acid, ofloxacin, sparfloxacin, novobiocin, coumermycin A1, clorobiocin, coumermycin;trovafloxacin

Multi drug P - cloxacillin, oxacillin, ciprofloxacin, norfloxacin, erythromycin, tetracycline

Multi drug Q - erythromycin, cloxacillin, tetracycline, oxacillin, novobiocin, nalidixic acid, norfloxacin

Multi drug R - erythromycin, roxithromycin, lincomycin, telithromycin, clarithromycin, clindamycin, tylosin, spiramycin, azithromycin, dirithromycin, pristinamycin IA, quinupristin, pristinamycin IIA, madumycin II, griseoviridin;dalfopristin, pristinamycin IB, virginiamycin S2, vernamycin B-gamma, vernamycin C, patricin A, patricin B, ostreogrycin B3, oleandomycin

Multi drug S - erythromycin, roxithromycin, telithromycin, clarithromycin, azithromycin, dirithromycin, oleandomycin

Multi drug T - gentamicin C, tobramycin, gentamicin B, amikacin, kanamycin A, apramycin, neomycin

Multi drug U - neomycin, ribostamycin, kanamycin A, gentamicin B, paromomycin, lividomycin A, lividomycin B

Multi drug V - sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfacetamide, mafenide, sulfasalazine, sulfamethizole

Multi drug W - sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfacetamide, mafenide, sulfasalazine, sulfamethizole, dapsone

Multi drug X - sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfacetamide, mafenide, sulfasalazine, sulfamethizole, sulfamethazine

Multi Class A - amikacin;gentamicin C;tobramycin

Multi Class B - azithromycin;gentamicin;nitrofurantoin;oxacillin;spiramycin

Multi Class C - Carbapenems, Penams, Cephamycins, Cephalosporins

Multi Class D - cefalothin, chloramphenicol, tigecycline, ampicillin, tetracycline, rifampin

Multi Class E - Cephalosporins, Penams, Tetracyclines, Glycylcyclines, Phenicol antibiotics, Rifamycins, Fluoroquinolones Quinolones Quinolines, Triclosan

Multi Class F - ciprofloxacin, tigecycline, chloramphenicol, rifampin, tetracycline, ampicillin, cefalothin

Multi Class G - clofazimine, ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, nalidixic acid, ofloxacin, sparfloxacin, trovafloxacin

Multi Class H - clofazimine, gatifloxacin, ciprofloxacin, levofloxacin, moxifloxacin, nalidixic acid, ofloxacin, sparfloxacin, novobiocin, coumermycin A1, clorobiocin, coumermycin, trovafloxacin

Multi Class I - cloxacillin, oxacillin, ciprofloxacin, norfloxacin, erythromycin, tetracycline

Multi Class J - erythromycin, cloxacillin, tetracycline, oxacillin, novobiocin, nalidixic acid, norfloxacin

Multi Class K - Fluoroquinolones Quinolones, Aminocoumarin antibiotics

Multi Class L - Fluoroquinolones Quinolones, Cephalosporins, Penams, Cephamycins

Multi Class M - Fluoroquinolones Quinolones, Macrolides, Penams

Multi Class N - Macrolides, Aminoglycosides, Nitrofurans, Penams

Multi Class O - Multiple antibiotic resistance, Tetracyclines, Quaternary ammonium compounds

Multi Class P - Nucleoside antibiotics, Phenicol antibiotics, Lincosamides, Fluoroquinolones Quinolones Quinolines, Acridine dye

Multi Class Q - Penams, Carbapenems, Cephamycins, Cephalosporins, Monobactams, Tetracyclines, Rifamycins, Phenicol antibiotics, Glycylcyclines, Fluoroquinolones Quinolones, Triclosan

Multi Class R - Penams, Cephamycins, Cephalosporins, Fluoroquinolones Quinolines, Macrolides, Tetracyclines

Multi Class S - Penams, Cephamycins, Cephalosporins, Monobactams, Tetracyclines, Rifamycins, Phenicol antibiotics, Aminocoumarin antibiotics, Glycylcyclines, Fluoroquinolones Quinolines, Triclosan, Macrolides, Glycylcyclines

Multi Class T - Penems, Penams, Carbapenems, Cephamycins, Cephalosporins, Monobactams, Tetracyclines, Peptide antibiotics, Sulfonamides, Diaminopyrimidines, Macrolides, Phenicol antibiotics, Aminocoumarin antibiotics, Fluoroquinolones Quinolones

Multi Class U - Penems, Penams, Carbapenems, Cephamycins, Cephalosporins, Monobactams, Tetracyclines, Rifamycins, Phenicol antibiotics, Glycylcyclines, Fluoroquinolones Quinolines, Triclosan

Multi Class V - sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfacetamide, mafenide, sulfasalazine, sulfamethizole, dapsone

Multi Class W - tigecycline, chloramphenicol, rifampin, tetracycline, ampicillin, cefalothin

Multi Class X - trimethoprim, brodimoprim, tetroxoprim, iclaprim