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Whole-Genome Sequencing of Multi-Drug Resistant Escherichia coli in Farm Animals and Unraveling Antibiotic Resistance Mechanisms and Public Health Risks

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Abstract | Upon account for its involvement with antimicrobial resistance (AMR), the growing frequency of multidrug-resistant (MDR) Escherichia coli within agricultural animals poses a serious danger to both veterinary, and public health. Alongside an emphasis on genomic composition, resistance determinants, and the function of mobile genetic elements (MGEs) within the dissemination of resistance genes, this work characterized MDR E. coli isolates from dairy cattle using whole-genome sequencing (WGS). The genomic comparison between isolates demonstrated substantial genetic diversity because some bacteria used chromosome modifications for sensitivity regulation while others possessed resistance genes mphA and blaCTX-M-55 existing on plasmids. There was a highest resistance against imipenem (IMP) at 60% while phenotypic testing indicated that multiple antibiotic resistance occurred in 22.9% of the isolates, shown resistance to gentamicin (GM) and tetracycline (TET). These antibiotics displayed most effectiveness against E. coli isolates (>65%). Evolutionary paths took diverse directions from selection forces which resulted in isolates forming different clade groups based on their resistance profile compositions. Horizontal gene transfer (HGT) showed continuous activity due to the presence of a large supplementary genetic complex that includes integrons and transposases and prophages which emerged from pangenome research. The isolated organisms contained at least one of the three primary resistance mechanisms which included efflux pumps β-lactamases and aminoglycoside-modifying enzymes. Improved genomic surveillance of livestock facilities must become an immediate priority to detect new AMR developments because it lowers the risks of zoonotic hazards. The first vital actions against MDR E. coli agricultural dissemination require antibiotic stewardship programs together with checked antimicrobial utilization on farms alongside development of treatment alternatives containing efflux pump inhibitors and bacteriophage therapy.

Keywords | Multidrug-resistant *E. coli*, Antimicrobial resistance, Whole-genome sequencing, β-lactamases, Phylogenetic analysis, Pangenome

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INTRODUCTION

Globally, dairy farming is important for food production by supporting agricultural sustainability, and economic stability and providing necessary nutrients coming from milk, and dairy products. Providing proteins, vitamins, and minerals essential for human health, dairy cattle help much to provide food security (Abdelfattah *et al*., 2021). Beyond just nutrition, dairy farming provides commerce, and jobs, especially within rural areas where cattle farming still generates the major source for revenue. through means for organic manure generation, which improves soil fertility, and supports sustainable agricultural methods, the sector also helps to contribute environmental sustainability. However, the demanding character for dairy farming has also brought significant difficulties, especially alongside relation to the development for antimicrobial resistance (AMR) among bacterial populations within cattle surroundings (Benkova *et al*., 2020).

Animal and human health depends on the analysis of Escherichia coli (E. coli) as a source of AMR genes in dairy cattle that presents significant health risks. The facultative anaerobic bacterium E. coli naturally exists in human and animal digestive tracts and manifests as two main pathotypes called STEC and ExPEC E. coli which represent important zoonotic infection agents (Algammal *et al*., 2020). Medical experts remain uncertain about new multidrug-resistant (MDR) E. coli strains which show resistance to antibiotic classes such as β-lactams and fluoroquinolones and aminoglycosides as described by Chen *et al*. (2019). The dissemination of AMR genes across E. coli occurs primarily through mobile genetic elements referred to as plasmids, transposons, and integrative conjugative elements according to Durrant *et al*. (2020). Misuse and abuse of antibiotics in the veterinary sector are primarily the causes of the pandemic of MDR E. coli in dairy farms (Kareem *et al*., 2023; Aziz *et al*., 2023; Awosile and Agbaje, 2021). Application of antimicrobial drugs therapeutically and prophylactically in bovine farming creates selection pressure that maximizes surviving and doubling the drug-resistant population of bacteria. Also contributing to the issue is horizontal transfer to the environment by faeces for resistant bacteria, enabling resistance determinants to expand further in microbial populations (Dionisio *et al*., 2023; Ali *et al*., 2024; Al-Sailawi *et al*., 2024; Mohsen *et al*., 2024). Evidence supports the demand for genomic surveillance, stewardship response on antimicrobials to add determinants of resistance to MDR E. E. Escherichia coli from crops' soil by the Comprehensive Antibacterial Resistance Database (CARD) (Alcock *et al*., 2023).

The spread of AMR genes receives special contribution from mobile genetic elements (MGEs). Integrons and transposons and MGE bacteriophages facilitate resistance determinant spread through short enough periods for bacteria to adapt to antibiotic utilization pressures (Brown *et al*., 2022). Plasmids contained extended-spectrum β-lactamase genes (ESBLs) blaCTX-M and blaTEM together with the measures directed at decreasing cattle disease resistance (Hall *et al*., 2021). WGS analysis revealed the simultaneous presence of resistance genes with highly mobile plasmids in agricultural environments where determination of antibiotic resistance determinant transfer across species was established (Alcock *et al*., 2020). The research sequenced whole genomes of multidrug-resistant (MDR) Escherichia coli isolates from dairy cattle to tackle increasing antimicrobial resistance (AMR) in dairy farming. Using whole-genome sequencing (WGS), we investigated the genomic features of MDR E. coli isolates while comparing how AMR genes distribute across plasmids rather than chromosomal DNA. Mobile genetic elements play a main role in enabling resistance gene transfer for which the AMR propagation rate accelerates according to this study. Research focused on assessing potential public health consequences of MDR E. coli stands among the principal issues alongside its food transmission risks. The paper examines environmental factors affecting AMR gene transmission and persistence within dairy farming establishments. The findings provide essential evidence to develop interventions for stopping MDR infection emergence in dairy production systems and demonstrate which genetic traits promote resistance.

MATERIALS AND METHODS

Study Design and Sample Collection

The researchers performed this cross-sectional study to evaluate both the prevalence and genetic aspects of multidrug-resistant (MDR) Escherichia coli strains isolated from dairy cattle in Iraq. The researchers took caution to prevent environmental contamination by obtaining aseptic fecal samples from dairy cows right after they defecated. The researchers used sterile 50-ml centrifuge tubes properly maintained at 4°C to transfer each sample then moved them to the laboratory under the established cold-chain protocols (Ju and Willing, 2018). Dairy farms receiving a documented history of substantial antibiotic treatment formed the basis of targeted sampling for MDR profiling with the aim to obtain relevant clinically significant and MDR strains.

Bacterial Isolation and Identification

Selective culture medium including MacConkey agar, Eosin Methylene Blue (EMB) agar, and Luria-Bertani (LB) agar helped to isolate E. coli. to guarantee 100% colony isolation, we used the streak plate approach. Colonies displaying characteristic E. coli shape (pink upon MacConkey agar, metallic green upon EMB agar) were chosen for

further biochemical, and molecular validation subsequent to 24 hours for incubation for 37°C (Liu *et al.*, 2021). Using universal primers 27F, and 1492R, 16S rRNA sequencing used to be used within molecular identification to amplify the gene throughout all nine variable regions (Liu *et al.*, 2022). Following optimal thermal cycling conditions, and a 25-µl volume, PCR reactions were conducted; gel electrophoresis used to be furthermore used to verify the amplification. to confirm species identification, the sequences matched NCBI's BLAST database.

Antimicrobial Susceptibility Testing

Following Clinical, and Laboratory Standards Institute (CLSI) recommendations, antimicrobial resistance patterns were evaluated using Kirby-Bauer disk diffusion technique. The research included fourteen medications coming from many categories for antimicrobial agents (Le Terrier *et al.*, 2024). Growing E. coli isolates on Mueller-Hinton agar, antibiotic-impregnated disks were arranged for specified spacing prior to incubation for 37°C for 16–18 hours. CLSI breakpoints guided measurement, and interpretation for the zone for inhibition around each disk. Each isolate's multiple antibiotic resistance (MAR) index used to be computed; the isolates were categorized like sensitive, intermediate, or resistant.

Whole-Genome Sequencing and Bioinformatics Analysis

Two very resistant MDR E. coli strains were chosen for whole-genome sequencing (WGS) utilizing the Illumina NovaSeq 6000 platform alongside a paired-end 150 bp read approach. The Qiagen DNA extraction kit used to be used to extract genomic DNA; Nextera XT DNA library preparation kit used to be used to create sequencing libraries (Lu *et al.*, 2024). FastQC (Andrews *et al.*, 2022) used to be used for quality control subsequent to sequencing; Trimmomatic furthermore removed adapters, and trimmed reads. Prokka v1.14.6 used to be used for genome annotations; SPAdes v3.15.5 used to be used for de novo genome assembly. RGI v6.0.3 discovered antimicrobial resistance genes (ARGs) against the Comprehensive Antibacterial Resistance Database (CARD) (Alcock *et al.*, 2023).

Phylogenetic and Pangenome Analysis

Using IQ-TREE2 using the Maximum Likelihood approach, and 1,000 bootstrap repetitions for accuracy (Minh *et al.*, 2020) phylogenetic connections between MDR E. coli isolates were deduced. Using Roary v3.13.0, pangenome analysis used to be conducted classifying genes into core, accessory, and unique categories (Kalyaanamoorthy *et al.*, 2017). FastTree v2.1.11 produced evolutionary trees; iTOL v5.0 (Letunic and Bork, 2021) visualizations were created coming from these trees. Evolutionary models for the dataset were tuned using Model Finder (Hoang *et al.*, 2018).

Identification for Mobile Genetic Elements (MGEs)

PlasmidFinder, ISfinder, and MobileOG-db were used to examine the function for mobile genetic elements (MGEs) within horizontal gene transfer (HGT) within order to locate plasmids, insertion sequences, and prophage areas (Brown *et al.*, 2022). Using VirSorter, and PhiGARo, the presence for integrons, and transposons used to be evaluated; recombination hotspots (Hiraizumi *et al.*, 2024) were defined using MGEfinder.

Extended-Spectrum Beta-Lactamase (ESBL) Gene Analysis

Genetic research on blaCTX-M-55 and blaTEM-1 genes established their basic evolutionary patterns. The MUSCLE tool served previously as a multiple sequence alignment (MSA) method but then phylogenetic trees were constructed through the IQ-TREE2 software (Minh *et al.*, 2020). The researcher evaluated confidence levels through bootstrap values which were calculated by performing one thousand repetitions. Scientists previously accessed NCBI public databases through which they conducted comparative genomic analyses.

Data Visualization and Statistical Analysis

The analysis used Python (Pandas, NumPy, Matplotlib) means for descriptive summary representations while iTOL (Letunic and Bork, 2021) generated both phylogenetic and pangenome images. The heatmap generated by Seaborn revealed clusters of antimicrobial resistance genes which served as factors to identify resistance determinant candidates. The following Table 1 outlines the key experimental procedures;

RESULTS AND DISCUSSIONS

Phenotypic Analysis for Antibiotic Resistance

Among the tested antibiotics E. coli isolates demonstrated sensitivity to 51.1% of the strains while 26% displayed intermediate resistance and antibiotic resistance occurred in 22.9% of cases (Figure 1A). The MDR classification applied to 26 out of 53 total E. coli isolates. Figures 1B and 1C revealed gentamicin (GM) and tetracycline (TET) as the most effective antibiotics as they yielded sensitivity rates at 65% while imipenem (IMP) showed maximal resistance at 60%. The relationship between ampicillin (AMP) resistance patterns matched those of trimethoprim/sulfamethoxazole (SXT) resistance patterns (r = 0.85). This indicates both antibiotics exhibited similar resistance mechanisms. The strength of resistance mechanisms showed low or no connection in antibiotic pair gentamicin (GM) and levofloxacin (LEV) therefore indicating each resistance mechanism operated autonomously (Figure 1D).

Table 1: Overview for methodology.

Methodology Step	Description
Study Design, and Sample Collection	Cross-sectional study alongside fecal sample collection coming from dairy cows, stored within sterile conditions, and transported for analysis.
Bacterial Isolation, and Identification	Cultured upon MacConkey, EMB, and LB agar; confirmed via 16S rRNA sequencing, and BLAST analysis.
Antimicrobial Susceptibility Testing	Kirby-Bauer disk diffusion method following CLSI guidelines; resistance categorized into susceptible, intermediate, or resistant.
Whole-Genome Sequencing, and Bioinformatics Analysis	WGS using Illumina platform; quality filtering alongside FastQC; assembly via SPAdes; annotation alongside Prokka; AMR genes identified using RGI against CARD.
Phylogenetic, and Pangenome Analysis	IQ-TREE2 alongside Maximum Likelihood method, and bootstrapping (1,000 replicates); pangenome analysis alongside Roary.
Identification for Mobile Genetic Elements (MGEs)	Detection for plasmids, insertion sequences, and prophage regions using PlasmidFinder, ISfinder, and MobileOG-db.
Data Visualization, and Statistical Analysis	Statistical analysis using Python libraries (Pandas, NumPy, Matplotlib); phylogenetic trees, and pangenome visualizations alongside iTOL.

Three separate groups—sensitive, intermediate, and resistant strains—were found through hierarchical clustering for isolates based upon their median antibiotic response scores within Figure 1E; Fascinatingly, several MDR isolates fell within the susceptible group upon account for their general sensitivity to most antibiotics, hence underscoring the variety for resistance phenotypes.

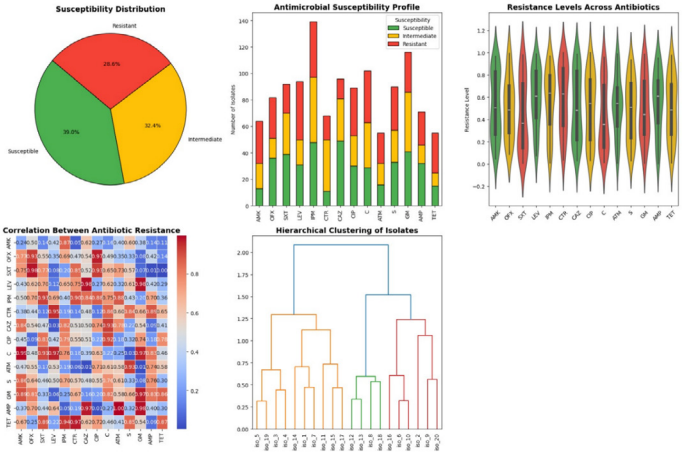

Figure 1: Phenotypic resistance profiles.

Evidence supports earlier research about carbapenem-resistant E. coli because imipenem resistance demonstrates that the microorganisms produce carbapenemases and alter porins to limit penetration of antibiotics (Shariati *et al.*, 2022). Uddin *et al.* (2021) have shown that SXT resistance together with AMP resistance consistently coexists because these genes represent plasmid-mediated resistance mechanisms. Research results from the clustering study demonstrate wide variability within MDR isolates thereby proving the requirement for specific therapeutic approaches for fighting particular resistance patterns.

Genome Assembly and Annotations

The Plasmid30 carried multiple resistance genes while having a length for ~218 kb. The genomic size of E.coli_45 amounted to ~4.99 Mb with a GC base content reaching 50.57%. Genomic analysis revealed 5,076 genes distributed among hypothetical proteins as well as virulence-associated genes. The analysis of antibiotic resistance and pathogenicity in multidrug-resistant (MDR) Escherichia coli was conducted through the whole-genome sequencing (WGS) of two selected isolates E.coli_30 and E.coli_45. The examined genomes exhibited contrasting features for size dimensions as well as gene count distributions and mobile genetic element (MGE) presence which included plasmids and insertion sequences and antibiotic resistance genes. Figure 2 presents circular genome maps that depict the genomic arrangements of E.coli_30 together with its Plasmid30 and E.coli_45. The sequencing genomes show annotation for critical genomic data which consists of GC content, GC skew and coding sequences (CDS) and antibiotic resistance genes (CARD database hits) along with ribosomal RNA (rRNA), transfer RNA (tRNA) and repetition regions. The circular genomic layouts of these elements present data about structural and functional divisions in each isolated bacterial strain.

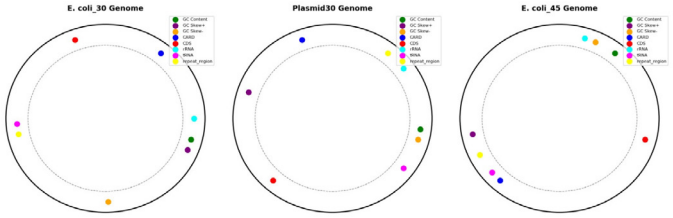

Figure 2: Genomic features.

Whereas E.coli_45 showed a somewhat smaller genome (~4.99 Mb) alongside a GC content for 50.57%, the de novo assembly for E.coli_30 produced a total genome size for ~5.12 Mb alongside a GC content for 50.92%. These values match previously sequenced clinical, and environmental isolates (Romeo *et al.*, 2023), and are within the

predicted range for E. coli. E.coli_30 (4,767) has less annotated genes than E.coli_45 (5,076), maybe suggesting more non-coding areas or mobile genetic elements within the later strain. Especially Plasmid 30 (middle genomic map) has many antimicrobial resistance genes shown through CARD database hits. for horizontal gene transfer (HGT), this plasmid is a vital vector, that helps resistance characteristics to be transmitted among bacterial populations (Vinayamohan *et al.*, 2022). Plasmid 30's presence for IncFIB, and IncFII replicons supports even further its function within gene mobilization, and persistence inside E. coli strains. discrepancies within mobile genetic elements (MGEs) might help to explain the discrepancies within genome size, and content between E. coli 30, and E. coli 45. More putative proteins, and virulence-associated components within E. coli-45 point to more genomic flexibility, and adaptation potential within clinical situations (Sarowska *et al.*, 2019). Further suggesting genome recombination events, variations within GC skew patterns might have helped these organisms evolve beneath selective antibiotic pressure (Touchon *et al.*, 2020).

Table 2: Comparative analysis for resistance mechanisms within E. coli_30, and E. coli_45.

Feature	E. coli_30	E. coli_45
Efflux pumps	RND, MFS, SMR, ABC, plasmid-borne efflux	RND, MFS, SMR, ABC, broader diversity
Antibiotic target *alt*eration	Quinolone target protection (*qnrB*, plasmid-borne)	Broader mutations (*soxR, marR*), increased fluoroquinolone resistance
Antibiotic inactivation	Macrolide phosphotransferase (*mphA*) via plasmid	Aminoglycoside-modifying enzymes (*APH(3')-IIa, APH(3")-Ib*)
Plasmid	Present; contributes to MDR	No plasmid detected
Aminoglycoside resistance	Limited inactivation mechanisms	Multiple aminoglycoside-modifying enzymes
β-Lactam resistance	Absent	*blaTEM-1*, *blaCTX-M-55*, ESBL-producing

Antimicrobial Resistance Mechanisms

The Resistance Gene Identifier (RGI) analysis detected the complete count of antimicrobial resistance genes in both genomes. The two isolates shared the entire set of resistance-nodulation-cell division (RND) family of gene efflux pumps which displayed resistance against tetracyclines fluoroquinolones and cephalosporins. Plasmid 30 contained the mphA gene besides genomic elements that are not naturally found in E. Coli-45. The antibiotic pumps in bacterial cells evacuate drugs from their environment which reduces the effectiveness of treatment thus enabling multidrug resistance (Tambat *et al.*, 2022). Plasmid 30 demonstrates how plasmid-mediated antimicrobial resistance genes play a major role in spreading AMP spread through mobile genetic elements (MGEs) according to Romeo *et al.* (2023). The observed findings support international research which demonstrates plasmid involvement in antimicrobial resistance development (Sarowska *et al.*, 2019). The Resistance Gene Identifier (RGI) analysis identified multiple antimicrobial resistance (AMR) genes which used distinct types of protection mechanisms in both E. coli_30 and E. coli_45. The acrA, acrB and mdtA RND family efflux pumps in both isolates enabled resistance against fluoroquinolones, cephalosporins and tetracyclines. E. coli_30 expressed a greater number of resistance elements on Plasmid 30 despite lacking the macrolide phosphotransferase genes (mphA) which Plasmid_45 did not contain. Research shows that E. coli_45 contain both APH(3′)-IIa and APH(3′)-Ib enzymes which demonstrate better aminoglycoside resistance capacity than E. coli_30 alone holds.

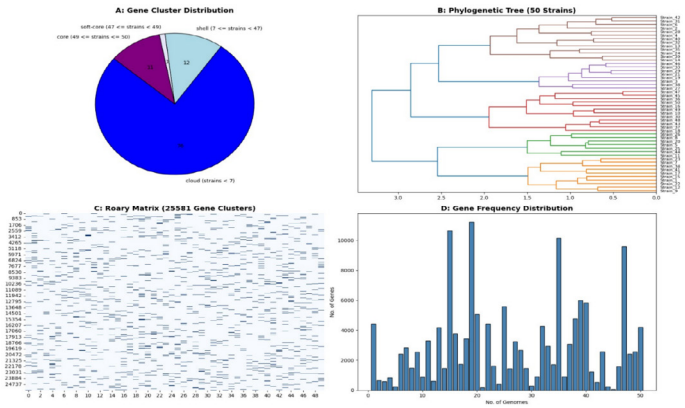

Figure 3: Phylogenetic tree.

E. coli_30's plasmid-mediated resistance elements—especially the macrolide resistance gene mphA—indicate, that horizontal gene transfer is clearly important within its resistance profile (Romeo *et al.*, 2023). through contrast, E. coli_45 seems to rely more upon chromosomal mutations, and intrinsic resistance mechanisms, including a wider range for β-lactam resistance genes (blaCTX-M-55, blaTEM-1), which have been linked within extended-spectrum β-lactamase (ESBL) production, and enhanced β-lactam resistance (Sarowska *et al.*, 2019). The higher variety for efflux pumps within E. coli_45 might also help to explain its capacity to release a larger spectrum for antibiotics, therefore lowering the treatment effectiveness (Tambat *et al.*, 2022). The results underline the need for focused treatments like they clearly show a difference between plasmid-mediated, and chromosomal resistance mechanisms. Efflux systems—especially RND, and ABC transporters—suggestive for inhibitors aiming for these

systems might be a workable approach to reduce multidrug resistance (Shariati *et al*., 2022). Furthermore, the lack for a plasmid within E. coli_45 increases the likelihood, that its resistance features would be less likely to be passed horizontally than within E. coli_30, which possesses mobile genetic components (Vinayamohan *et al*., 2022). Developing more efficient antimicrobial treatments depends upon an awareness for these resistance mechanisms, especially within relation to bacterial illnesses resistant to many drugs.

Virulence-Associated Genes

The virulence gene analysis identified Type I fimbriae (fimA, fimB, and fimC) responsible for adhesion together with the enterobactin siderophore systems essential for iron uptake as factors shared among the two genomic sequences. Still the analysis identified specific virulence structures.

The E.coli_30 strain contains iroB and iroC salmochelin siderophore genes that allow it to acquire more nutrients. The virulence elements within E.coli_45 include the T6SS genes tssA and hcp1 that enable competition against other bacteria as well as facilitate interactions with human hosts. Salmochelin siderophores supply advantageous traits to these isolates for surviving in restricted iron conditions that exist in host tissues (Sora *et al*., 2021). The T6SS system enables E. coli strains to achieve superior interbacterial competition suitability to the most harmful E. coli species (Shariati *et al*., 2022).

Phylogenetic Relationships and Evolutionary Insights

Laboratory tests demonstrated that E.coli_30 had ST3579 sequence type through MLST analysis and E.coli_45 matched ST1121. Testing showed that all isolates generated distinct phylogenetic groups according to Figure 3A. Research using 50 bacterial strains identified a fundamental genomic area that incorporated transposases and presented integrons together with 2,755 vital genetic elements. In phylogenetic research the study focused on using whole-genome sequencing data to analyze evolutionary relationships of E.coli isolates. Different presentations in Figure 3 present data about (A) gene cluster distribution followed by (B) phylogenetic analysis of 50 E. coli strains and (C) the Roary matrix with 25,581 gene clusters alongside (D) gene frequency distribution histogram. The database shows four gene cluster divisions that organize core genes and soft-core genes together with shell genes and cloud genes (Figure 3A). The core genome keeps essential functional genes that many strains have in common while accessory genes from shell and cloud segments generate genomic variations and adaptation mechanisms. The main reason for genetic variations in strains comes from cloud genes that exist in less than seven strains while HGT represents a conventional way for strains to acquire new genes (Vinayamohan *et al*., 2022). Figure 3B represents the evolutionary relationship between isolates through graphical display. Multiple E. coli clades form from the mixture of evolutionary forces and environmental elements leading to separate phylogenic groupings. The evolutions of E. coli_30 and E. coli_45 show separate developmental patterns possibly affected by antimicrobial resistance genes (AMR genes) and mobile genetic elements (MGEs) based on Touchon *et al*. (2020). Genetic flexibility stands as a strong E. coli trait due to the Roary matrix (Figure 3C) revealing gene absence and presence in studied strains. The need for bacterial evolution depends on genomic flexibility because accessory genes such as transposases and integrons and antibiotic resistance genes pattern the way Uddin *et al*. (2021) explain it. In Figure 3D researchers show the distribution pattern of genes that exist independently between E. coli bacterial genomes. Genomic evolution in growing bacterial communities becomes visible through the range of rare genes whereas the distribution peaks reveal which genes show the highest level of consensus.

Phylogenetic analysis demonstrates that E. coli_30 and E. coli_45 belong to different evolutionary lines since they possess distinct genetic makeup faced with different natural selection processes. The large number of cloud genes indicates that transferred genes serve as key components for bacterial evolution both by facilitating antimicrobial resistance gene spreading (Uddin *et al*., 2021). The validity of MLST pattern assignment stands validated through its ability to demonstrate how different bacterial isolates cluster into sequence types (STs) that stay together (Tamura *et al*., 2021). Bacterial genetic adaptability stems from various accessory genes in the pangenome database which allows E. coli quick strain adaptation and antimicrobial stress response capabilities. The experimental findings in combination with this process reveal that plasmids and phages together with insertion sequences (Romeo *et al*., 2023) normally accept virulence and resistance genes that derive from pathogenic E. coli strains. The continuous practice of genomic surveillance provides crucial benefits by turning potentially dangerous new antibiotic resistance threats into detectable phenomena. The observed genetic diversity shows that recombination together with mobile genetic elements generates most genomic variations present in E. coli. Fundamental evolutionary traits of multidrug-resistant E. coli isolates link to genome stability of core elements in contrast to the independent modifications of extra genetic components. Research based on genome analysis demonstrates urgent necessity to combat antibiotic resistance because MGE and virulence-associated genes exist with antibiotic resistance determinants (Vinayamohan *et al*., 2022).

Mobile Genetic Elements

The two E. coli genomes contain insertion sequences from the IS110 family along with transposons from the Tn3 family together with integrons and prophages among other mobile genetic elements. The analysis demonstrated E. coli_30 with five prophage areas but E. coli_45 contained eleven prophages along with distinctive lysogeny and horizontal gene transfer (HGT) purposes. The gene acquisition capability together with transmission functions of MGEs serve as crucial evolutionary drivers for bacteria (Starikova *et al*., 2020). The large number of MGEs contained in these genomes defines them as virulence factor reservoirs (Vinayamohan *et al*., 2022) and antimicrobial resistance gene hosts. Bacterial fitness complexity arises from prophages whereas these elements enable lysogenic conversion to enhance bacterial fitness which succeeds in various environments (Yang *et al*., 2021).

Prospects

HD-1 shows different resistance mechanisms which this study discovers in plasmids and chromosomal mutations as well as through efflux pumps. The dissemination of genes conferring antibiotic resistance occurs through the mobile elements integrons together with transposons and prophages. Phylogenetic assessment shows varieties of isolates surpass all previous expectations while climate variables and medicinal drug administration shape the way organisms evolve. E. coli shows great versatility in agricultural environments because its additional genetic variants provide resistance to antibiotics. Antimicrobial stewardship principles and gene monitoring systems represent essential components which research establishes for preventing MDR bacteria transmission by animal dissemination. The healing science of One Health serves as the foundational approach through which healthcare authorities should manage the complex environment-genetics relationship around antibiotic resistance. Multiple essential steps are needed to stop the growth of multidrug-resistant (MDR) Escherichia coli in cattle environments. Genomic surveillance has to become a top priority since it will help detect E. coli isolates through whole-genome sequencing to identify resistance mechanisms and track their evolution. These precise solutions and focused containment approaches will become available due to this monitoring system. The prevention of extreme antibiotic use on farm animals requires antimicrobial stewardship programs. Promoting alternative therapies, and controlling the use of antibiotics will serve to reduce the selection pressures resulting in antimicrobial resistance (AMR). To stop the development for resistant germs, biosecurity policies within farms should also be strengthened alongside better cleanliness practices, enhanced waste management, and tighter control atop animal-to--animal, and human-to-animal contacts. Developing new treatment drugs, like efflux pump inhibitors, and antimicrobial compounds, that interfere alongside horizontal gene transfer (HGT), helps to target efflux pumps, and mobile genetic elements (MGEs), thereby battling MDR strains. MDR E. coli has zoonotic consequences, hence public health integration is rather important. Improved cooperation across the veterinary, agricultural, and medical health sectors will enable a complete One Health strategy to control AMR to be developed. Furthermore, investigated should be other therapeutic modalities such probiotics, bacteriophage treatment, and antimicrobial peptides like possible replacements for traditional antibiotics within cattle treatment. to reduce selection pressure for resistance genes, more stringent rules upon the use for antibiotics within animal feed are ultimately required. Strong rules upon the use for antibiotics within agriculture help to drastically lower the possibility for AMR spreading within cattle herds, and maybe infecting people. Controlling MDR E. coli, and maintaining the effectiveness for present antibiotics will depend mostly upon these coordinated efforts.

CONCLUSIONS AND RECOMMENDATIONS

Whole-genome sequencing of a study of Iraqi dairy cattle revealed high multidrug-resistant E. coli prevalence (49.1%). Twenty six of 53 isolates were MDR and all had different resistance mechanisms, some plasmid derived (E.coli30) versus others chromosomally based (E.coli45). The phylogenetic analysis showed that the evolution was independent and the highly mobile prophages and integrons were involved in the transfer of resistance genes. The virulence genes are zoonotic, and difficult resistance profiles to ampicillin and trimethoprim/sulfamethoxazole cause a risk to public and veterinary health.The management needs more genomic surveillance, antimicrobial stewardship, enhanced farm biosecurity, and a One Health approach, which involves veterinary, environmental and public health. Alternative treatment, such as bacteriophages, antimicrobial peptides must be worked out and powerful regulation systems with on-reporting and educational sessions should be discovered. Precision veterinary medicine requires good farming practises such as vaccination, food, probiotics and international research and sharing of ideas to reduce resistance and to maintain the usefulness of antibiotics. effectiveness in the future.

ACKNOWLEDGEMENTS

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NOVELTY STATEMENT

The study is the first to analyze the whole-genome sequence of Iraqi dairy cattle E. coli infected by MDR, descending plasmid-mediated or chromosomal resistances and introducing new evolutionary trends at agricultural MDR strains.

AUTHOR'S CONTRIBUTIONS

Fayhaa Muhammad Najm planned the investigation and did isolation of bacteria. Antimicrobial tests were carried out by Hind Tahseen Ibrahim. Sampling was done by Raed amer Ali Alsahoo. Sequencing preparation was done by Ibrahim Ayad Jihad. Phylogenetic analysis has been under the supervision of Qais R. Lahhob. Bioinformatics analysis was done by Mustafa Mudhafar. Hasan Ali Alsailawi made the project coordination and the writing of the manuscript. Analysis of data was done by Mustafa Adnan Zaidan. The final manuscript has the stamp of approval of all the authors.

Conflict of Interest

There are no competing financial interests or conflicts of interest around this research presented by the authors.

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