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RESEARCH ARTICLE

Co-occurrence of Antimicrobial Resistance and Virulence Genes on Diverse Mobilomes in Dairy Farm Waste *Escherichia coli*: a Genomic Analysis

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ABSTRACT

Dairy farms waste carries multiple bacterial species particularly *Escherichia coli*, a potential reservoir and disseminator of antimicrobial resistance genes (ARGs) and virulence genes (VGs) through mobilomes. We performed whole-genome sequencing and bioinformatics analysis on 64 multidrug-resistant *E. coli* isolates from dairy farm waste in Gansu, China, to characterize and predict the co-occurrence of ARGs and VGs on mobilomes. Bioinformatics analysis revealed that key ARGs (*sul2*, *rmtB*, $bla_{\text{TEM-1}}$) and VGs (*traT*, *iutA*) were predominantly plasmid-associated. Notably, a single Tn*2* transposon carrying co-localized $bla_{\text{TEM-1}}$ (ARG) and *clpK* (VG) is direct evidence of convergence on mobile element. Prophage regions and genomic islands were also rich reservoirs, with *ompT* (VG) and *emrE* (ARG) found in 62.5% and 45.3% of isolates, respectively, within the prophage regions. Similarly, *fimH* (VG) and *rsmA* (ARG) genes were predominantly identified in 64.1% and 48.4% isolates within the genomic island regions. The convergence of ARGs and VGs associated on diverse mobilomes indicate their potential of dissemination within and between the bacterial pathogens of animal and human significance. Further, our findings highlight the urgent application of integrated One Health policies to mitigate the potential risks.

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INTRODUCTION

Antimicrobial resistance (AMR) is a global health emergency that jeopardizes the antibiotics efficacy and making it harder to treat illnesses that were curable earlier (Almansour *et al*., 2023). The rise of multidrug-resistant (MDR) bacteria (conferring resistance to at-least ≥ 3 classes of antibiotics) is the most threatening outcome of AMR, particularly at the human-animal-environment interface (One Health) which has been identified as a global challenge (Zinno *et al*., 2023; Madni *et al*., 2025). The emergence of MDR bacteria resulted in a considerable increase in the disease burden in terms of morbidities, mortalities, financial burden, and threat to food safety in the present era (Dong *et al*., 2022). Nowadays, the rigorous and unjustified usage of antimicrobials in livestock resulted in the development of AMR pathogens. The disposal of livestock waste in the environment is a chief concern and is considered as biotic pollution (Lawal *et al*., 2022; Shoaib *et al*., 2025a). Livestock waste especially of dairy farms contain multiple species of bacteria particularly *Escherichia coli* (*E. coli*), a commensal and pathogenic organism colonizing the animal gut and a potential reservoir of carrying multiple antimicrobial resistance genes (ARGs) and virulence genes (VGs) (Shoaib *et al*., 2025b). The dairy farm waste may contaminate the environmental sites of public health concern such as ground water, surface water, agriculture land, dairy products, and drinking water (Zhang *et al*., 2021). Moreover, it is an important reservoir of emergence and horizontal spread of ARGs and VGs through *E. coli* and other pathogens of public health

concern which may pose a serious threat to animal and human health.

E. coli can acquire ARGs and VGs through mobile genetic elements (MGEs) including plasmids, insertion sequences (ISs), transposons (Tns), prophages, and genomic islands (GIs) (Khedkar *et al*., 2022). The spread of acquired ARGs and VGs within and between the bacterial pathogens of humans, animals, and the environment concern *via* the mechanisms of horizontal gene transfer (HGT) (Jian *et al*., 2021; Li *et al*., 2025). The ISs such as predominance of IS*26* in *E. coli* isolates mediate the amplification of *bla*$_{\text{CTX-M}}$ genes (Wang *et al*., 2023a). The gene carrying MGEs (also called as transposons) facilitate the dissemination of ARGs through transposition (Brkljacic *et al*., 2022). Recent studies have shown that plasmids serve as efficient vectors for the propagation of ARGs. About 27 various plasmid incompatibility (Inc.) groups have been identified in *Enterobacteriaceae* including IncF, IncHI2, IncA/C, IncN, IncI, and IncL/M plasmids as predominant families which facilitate the spread of AmpC-cephalosporins, carbapenemases, plasmid-mediated quinolone resistance (PMQR), and extended spectrum beta-lactamases (ESBL) genes (Munir *et al*., 2025; Shoaib *et al*., 2025c). Moreover, the prophages are dormant, integrated form of a bacteriophage genome within the DNA of a bacterial host. Prophages act as hidden genetic reservoirs and mobile vectors for spread of ARGs and VGs (Liao *et al*., 2024). GIs as the large clusters of genes with a certain GC content and dinucleotide frequency inside a bacterial genome. Different genomic islands, such as pathogenicity island (carrying VGs), resistance island (carrying ARGs), metabolic island (carrying metabolic genes), and symbiosis island (carrying symbiotic genes). Among them, pathogenicity and resistance islands play a significant role in the spread of ARGs and VGs through HGT with the support of integrative conjugative elements (ICEs) (Saini *et al*., 2023). Therefore, the convergence of ARGs and VGs on different mobilomes can play an important in their dissemination in One Health settings.

China's expanding dairy industry is actively contributing to the spread of AMR through practices such as unjustified antibiotic usage and inappropriate dairy farm waste disposal allowing resistant isolates and genes to emerge and circulate through HGT within the production system and potentially reach the environment and humans (Xiong *et al*., 2024). Therefore, the present study was designed on the basis that the use of antimicrobials in the dairy production may contaminate its surrounding environment and dairy farm waste can be the potential carrier and dissemination driver of ARGs and VGs in *E. coli* through diverse mobilomes.

MATERIALS AND METHODS

Origin of bacterial isolates: A total of 64 bacterial isolates conferring co-resistance to aminoglycosides and beta-lactams were isolated from a total of 319 including feces (n=265) and sewage (n=54) samples from two S and X large dairy farms in Gansu province, China (Shoaib *et al*., 2024). Samples were collected in March (farms cleaning time) each year (2017-2019). Briefly, the collected samples were processed for bacterial isolation and purification on MaConkey agar followed by presumptive species identification by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (Bruker, Massachusetts, USA). The isolates exhibited resistance to at least three classes of antibiotics such as aminoglycosides, beta-lactams, and tetracyclines as declared in Shoaib *et al*. (2024) were recognized as MDR.

DNA extraction and whole genome sequencing: The bacterial DNA of all isolates was extracted using the TIANamp bacterial DNA extraction kit (Tiangen, China). The extracted DNA was subjected to quality check using the Nanodrop and agarose gel electrophoresis followed by sequencing library preparation using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) and finally short-read sequencing on Illumina Novaseq PE150 platform.

Data processing and bioinformatics analysis: The raw data was processed for low-quality reads and adaptor removal using the Trimmomatic v.0.39 (Bolger *et al*., 2014) and quality check by FastQC v.0.11.8 (Wingett and Andrews, 2018). The clean data was subjected to *de novo* assembly using the SPAdes v.4.2.0 (Bankevich *et al*., 2012). The assembled data (in the form of contigs) were subjected to predict the plasmid or chromosomal contigs carrying ARGs and VGs using the mlplasmids v2.1.0 (Arredondo-Alonso *et al*., 2018) with *E. coli* as model specie, minimum sequence length of 500 bp, and posterior probability of belonging to either plasmid or chromosomal contig at a score of >0.7. Contigs with posterior probability <0.7 were considered "unpredictable". The ARGs and VGs carrying contigs of these isolates were identified as described earlier (Shoaib *et al*., 2024). The mobile genetic elements such as ISs, Tns, ICEs and their relation with ARGs and VGs were identified using the MobileElementFinder v1.0.3 at 90% sequence similarity and 85% query coverage under the Center for Genomic Epidemiology (CGE) server (https://cge.food.dtu.dk/services/, accessed on February 25, 2024). IslandPath was used to identify genomic islands (Hsiao *et al*., 2003) and prophage regions were predicted by PHASTEST online tool (https://phastest.ca/, accessed on March 15, 2024) (Wishart *et al*., 2023) at score ≥90. The GIs and prophage regions were further used as query sequences in Resistance Gene Identifer v6.0.5 and VirulenceFinder v2.0.5 to identify associated ARGs and VGs at 90% Identity and 85% coverage under the Comprehensive Antibiotic Resistance Database and Center for Genomic Epidemiology (CGE) server (https://cge.food.dtu.dk/services/, accessed on April 10, 2024).

Data visualization: The graph data presented in this article was generated using the GraphPad Prism v10.5.0 (https://www.graphpad.com/) while the heatmap was constructed using the chiplot online (https://www.chiplot.online/) and edited through the Inkscape v1.4.3 (https://inkscape.org/).

RESULTS

Predicted plasmid and chromosome associated ARGs and VGs: The genomic prediction analysis of ARGs revealed that *sul2*, *rmtB*, *aph(3")-Ib*, *aph(6)-Id*, *bla*$_{\text{TEM-1}}$, *floR*, and *tet(A)* were found to be highly associated with plasmids. The other ARGs such as *sitABCD*, *fosA3*, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-55}}$, *aac(3)-IId*, *aac(3)-IV*, and *aadA2*

were predicted to be associated with chromosome or unpredictable (Fig. 1). Similarly, the genomic prediction of VGs revealed that *traT*, *traJ*, *anr*, *iutA*, and *iucC* genes were predominately predicted in plasmid contigs while *ompT* gene was predicted in both plasmid and chromosomal contigs (Fig. 2). The *faeCDFJI* locus was only found to be predicted in the plasmid contigs of 2 isolates while the other VGs identified in chromosomal contigs or unpredictable are presented in Fig. 2. The prediction of ARGs in plasmid contigs may indicate their potential of dissemination through HGT.

Insertion sequences and transposons: A total of 66 MGEs belong to 19 different families were found in the genome of 64 MDR *E. coli* isolates at different percentage distributions. The identified MGEs belong to majorly 2 different categories; insertion sequences (ISs), and transposons (Tns). The most diversity of MGEs belonged to IS*3* family mobile elements. Among the ISs, MITEEc1 belong to IS*630* family were identified as the predominant (100%, 64/64) mobile element followed by IS*Ec1* belong to IS*As1* (92.2%), IS*609* belong to IS*605* family (73.4%), IS*Vsa3* of IS*91* family (68.7%), IS*30* (67.2%), IS*5075* (56.2%), IS*Ec17* (43.7%), IS*Ec38* and IS*Kpn8* (34.4% each). The percentage distribution of other ISs such as IS*6100*, IS*1006*, IS*Ec59*, IS*102*, IS*Kpn26*, IS*Ec31*, IS*3*, IS*629*, IS*421*, IS*4*, IS*Ec5*, IS*Cfr1*, IS*100*, IS*Ec9*, and IS*Ec78* were ranged from 10-30% (Table 1). However, the percentage of remaining ISs were found below 10% which are also listed in Table 1. Among the transposons, the unit transposon Tn*1000* was found predominantly in 43.7% (28/64) isolates while Tn*2* was only detected in single isolate carrying both $bla_{\text{TEM-1}}$ (ARG) and *clpK* (VG) genes, providing direct evidence for physical linkage of resistance and virulence on a mobile element. Moreover, eight different composite transposons were identified. Among the composite transposons, cn_5129_IS*Vsa3* was identified in 10.9% isolates, carrying *floR* gene. The composite transposon, cn_1670_IS*Ec1* (7.81%, 5/64), cn_4516_IS*3* and cn_12321_IS*629* (3.12%, 2/64 each) while the other 6 composite transposons were identified in single isolates (Table 1). Direct physical linkage of ARGs and VGs was confirmed only for Tn*2*, highlighting the need for higher-resolution technologies such as long-read sequencing to fully resolve co-localization patterns.

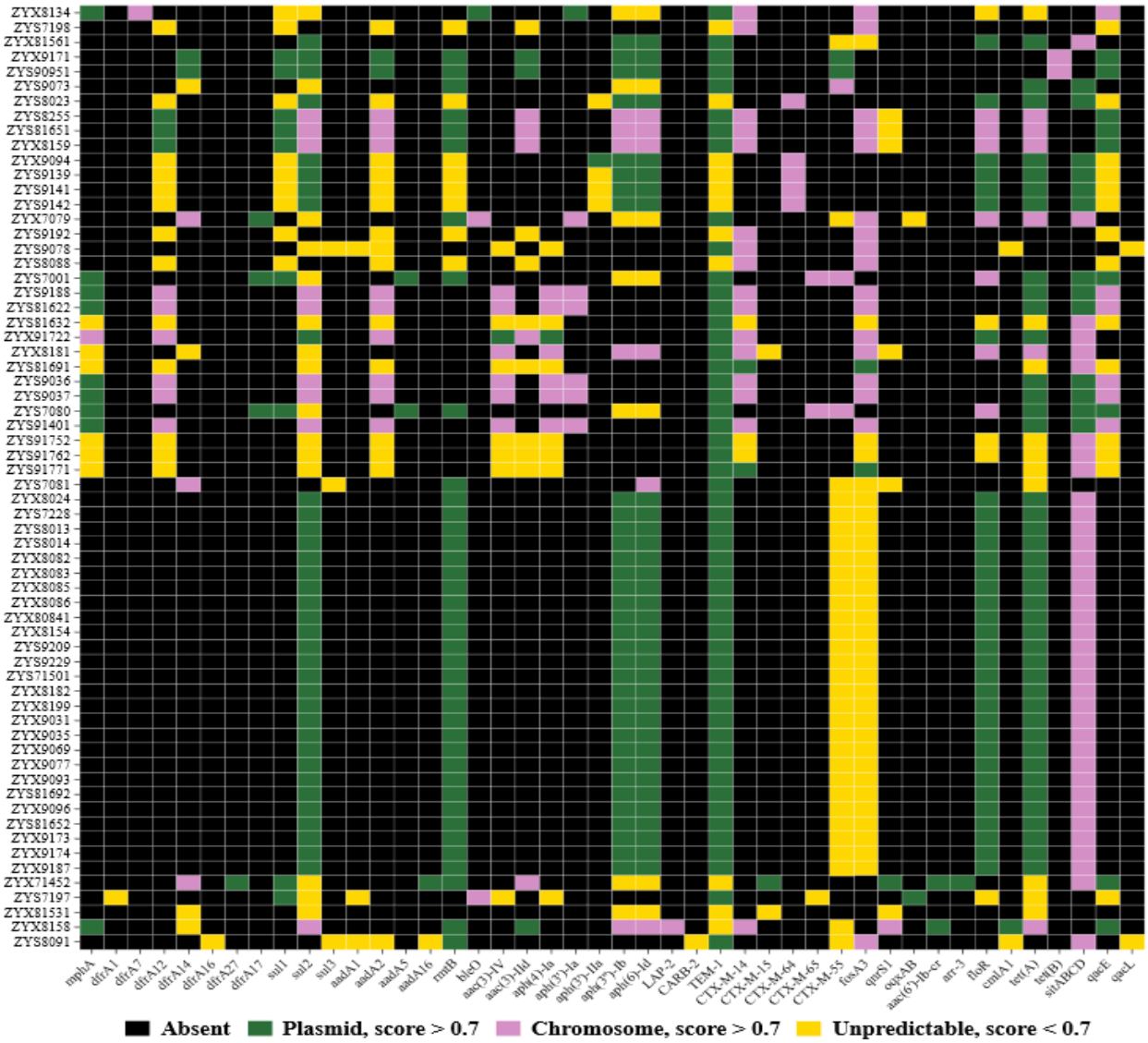

Fig. 1: Association prediction of ARGs with plasmid or chromosome based on mlplasmids (https://sarredondo.shinyapps.io/mlplasmids/, accessed on January 10, 2026).

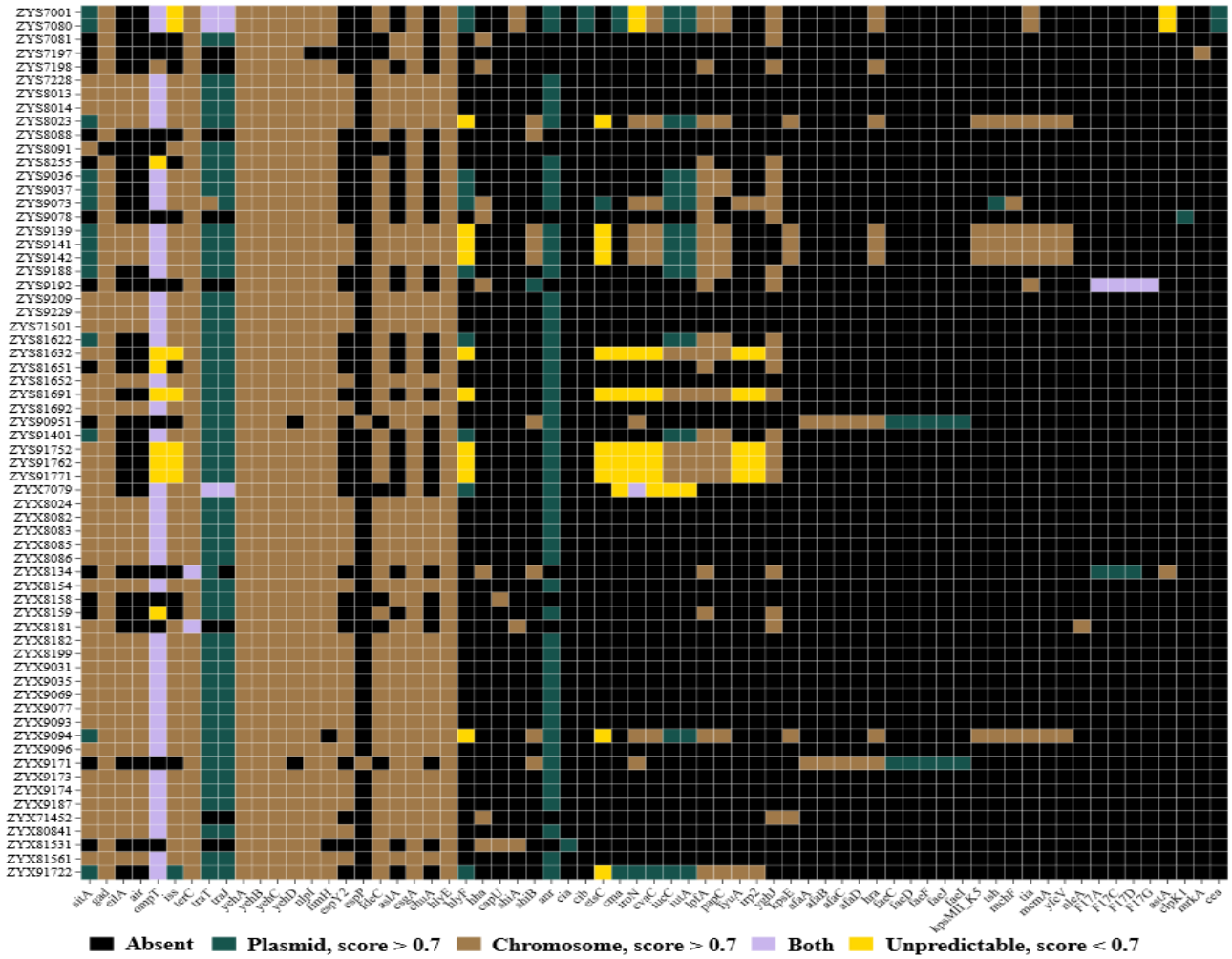

Fig. 2: Association prediction of VGs with plasmid or chromosome based on mlplasmids (https://sarredondo.shinyapps.io/mlplasmids/, accessed on January 10, 2026).

Table 1: Mobile genetic elements (insertion sequences and transposons) identified in 64 MDR *E. coli* isolates

Family	MGE	No. carrying	Percentage (%)	Family	MGE	No. carrying	Percentage (%)
IS*1*	IS*Ec30*	5	7.81		IS*Ec38*	22	34.4
	IS*6100*	13	20.3	IS*L3*	IS*Kox3*	4	6.25
	IS*1006*	8	12.5		IS*Pst2*	2	3.12
IS*6*	IS*Ec59*	12	18.7		IS*421*	9	14.1
	IS*26*	20	31.2	IS*4*	IS*Aba1*	1	1.56
	cn_12017_IS*26*	1	1.56		IS*4*	9	14.1
	IS*102*	16	25		IS*Sbo1*	2	3.12
	IS*903*	3	4.69	IS*91*	cn_5129_IS*Vsa3*	7	10.9
	cn_3011_IS*903*	1	1.56		IS*Vsa3*	44	68.7
IS*5*	IS*Kpn26*	12	18.7		IS*Ec1*	59	92.2
	IS*Vsa5*	1	1.56	IS*As1*	cn_1670_IS*Ec1*	5	7.81
	cn_4424_IS*Kpn26*	1	1.56		IS*Ec5*	7	10.9
	IS*5*	7	10.9	IS*605*	IS*609*	47	73.4
	cn_4424_IS*5*	1	1.56		IS*Ec46*	1	1.56
	MITEEc1	64	100	IS*30*	IS*30*	43	67.2
IS*630*	IS*Ec40*	2	3.12	IS*NCY*	IS*Esa1*	4	6.25
	IS*Ec33*	1	1.56		IS*Kpn21*	1	1.56
	IS*Kpn8*	22	34.4		IS*Ec45*	4	6.25
	IS*Kpn37*	1	1.56		IS*Ec81*	6	9.37
	IS*Sen4*	2	3.12	IS*110*	IS*Ec11*	1	1.56
	IS*Sfl10*	2	3.12		IS*5075*	36	56.2
	IS*Ec17*	28	43.7		IS*Sso6*	2	3.12
	IS*Ec31*	14	21.9		IS*Sfl8*	2	3.12
	IS*Ec52*	6	9.37	IS*1182*	IS*Cfr1*	14	21.9
IS*3*	IS*Ec10*	1	1.56	IS*21*	IS*100*	18	28.1
	IS*Eam1*	1	1.56	Unit Tn	Tn*1000*	28	43.7
	IS*911*	2	3.12		Tn2	1	1.56
	IS*Ehe3*	1	1.56	IS*1380*	IS*Ec9*	9	14.1
	IS*3*	13	20.3	IS*481*	IS*Ec18*	3	4.69
	cn_4516_IS*3*	2	3.12		IS*682*	1	1.56
	IS*629*	17	26.6	IS*66*	IS*Kpn24*	1	1.56
	cn_12321_IS*629*	2	3.12		IS*Ec47*	1	1.56
IS*Kra4*	IS*Kpn19*	2	3.12		IS*Ec78*	7	10.9

Prophage regions associated ARGs and VGs: The number of prophage regions identified in 64 MDR *E. coli* isolates were ranged from 2 to 19 (Fig. 3). A total of 32 different prophage types were detected in the whole genome of 64 *E. coli* isolates. Among the detected prophage regions, Entero_P4 and Entero_P88 were identified as the most predominant type (35.9%, 23/64 each), followed by Vibrio_12B12 and Escher_pro147 (31.2%, 20/64), Klebsi_4LV2017 (29.7%, 19/64), Escher_HK639 (14.1%, 9/64), Entero_mEp460 (12.5%, 8/64), and Shigel_SfII (10.9%, 7/64). The percentage distribution of other identified prophage regions ranged from 1%-10% (Table 2).

Table 2: Identification of prophage region types, their host, and percent distribution in 64 MDR *E. coli* isolates

Sr. No.	Phage type	Host	No. carrying	Percentage (%)
1.	Shigel_SfII	*Shigella*	7	10.9
2.	Escher_HK639	*Escherichia*	9	14.1
3.	Klebsi_4LV2017	*Klebsiella*	19	29.7
4.	Entero_BP_4795	*Enterobacter*	2	3.12
5.	Shigel_POCJ13	*Shigella*	1	1.56
6.	Entero_SfI	*Enterobacter*	1	1.56
7.	Escher_pro483	*Escherichia*	2	3.12
8.	Entero_P1	*Enterobacter*	2	3.12
9.	Salmon_118970_sal3	*Salmonella*	1	1.56
10.	Escher_500465_1	*Escherichia*	4	6.25
11.	Entero_DE3	*Enterobacter*	3	4.69
12.	Entero_P4	*Enterobacter*	23	35.9
13.	Vibrio_12B12	*Vibrio*	20	31.2
14.	Escher_pro147	*Escherichia*	20	31.2
15.	Entero_ES18	*Enterobacter*	2	3.12
16.	Entero_P88	*Enterobacter*	23	35.9
17.	Burkho_phiE255	*Burkholderia*	2	3.12
18.	Entero_HK629	*Enterobacter*	1	1.56
19.	Salmon_SSU5	*Salmonella*	4	6.25
20.	Escher_vB_EcoM_12474III	*Escherichia*	2	3.12
21.	Entero_mEp460	*Enterobacter*	8	12.5
22.	Pseudo_phiPSA1	*Pseudomonas*	1	1.56
23.	Entero_cdtIx	*Enterobacter*	1	1.56
24.	Entero_lambda	*Enterobacter*	4	6.25
25.	Burkho_BcepMu	*Burkholderia*	2	3.12
26.	Salmon_SPN3UB	*Salmonella*	5	7.81
27.	Escher_RCS47	*Escherichia*	1	1.56
28.	Entero_SfV	*Enterobacter*	2	3.12
29.	Escher_520873	*Escherichia*	1	1.56
30.	Shigel_Sf6	*Shigella*	1	1.56
31.	Cronob_ESSI_2	*Cronobacter*	1	1.56
32.	Salmon_SJ46	*Salmonella*	1	1.56

A total of 48 different ARGs were predicted to be identified in the prophage regions belong to multiple classes of antimicrobials. The most abundantly identified ARG was *emrE* (45.3%) confer resistance to macrolides by acting as efflux pump, followed by *mdtM* (14.1%) confer resistance to multiple antimicrobials, *aph(6)-Id* (10.9%) confer resistance to aminoglycosides by antibiotic inactivation, *sul2* and *tet(A)* (9.37% each) confer resistance to sulfonamides and tetracyclines respectively, *aph(3'')-Ib* (7.81%) confer resistance to aminoglycosides, *emrABR* complex, *mdtP*, and *H-NS* (6.25% each), *acrAB-tolC-marR*, *parC*, and *EC-13* (4.69%, 3/64 each) while the other ARGs were identified in single and two isolates (Table 3).

A total of 28 different VGs were identified in the prophage genome segments. Among them, *ompT* were predominantly identified in 62.5% (40/64) isolates, followed by *iss* (45.3%, 29/84), *fimH* (18.7%, 12/64), *hlyF* (10.9%), *aslA*, *anr*, *etsC*, *fyuA,* and *irp2* (9.37%, 6/64 each), *yehABCD* and *papC* (6.25% each), *terC*, *nlpI*, *traJ*, *hlyE*, *hra*, and *capU* (3.12%, 2/64) (Table 4). Collectively, these findings demonstrate that prophage regions in dairy farm waste *E. coli* serve as reservoirs for both ARGs and VGs, with efflux pumps and immune evasion factors being particularly enriched.

Genomic Island (GI) regions associated ARGs and VGs: The number of genomic islands identified in 64 MDR *E. coli* isolates were ranged from 5 to 20 (Fig. 4). The number of VGs identified in GIs were noted higher (n=18) as compared to ARGs (n=4). Among the ARGs, *rsmA* confer resistance to fluoroquinolone, diaminopyrimidine, and phenicol were identified in 48.4% (31/64) of isolates followed by *emrE* (7.81%), *mdtM* (4.69%), and *bla*$_{\text{CTX-M-55}}$ (3.12%). However, 15.6% (10/64) and 51.6% (33/64) of the *E. coli* isolates were not carrying with any of the VGs and ARGs in GIs, respectively (Fig. 5A), suggesting that in this dataset, GIs are not major reservoirs of antimicrobial resistance. In contrast, VGs were widely distributed across the GIs. Among the VGs, type 1 fimbrial adhesin *fimH* was identified as predominant in 64.1% (41/64) isolates followed by *yehAB* (50%), *yehCD* (34.4%), *papC* (21.9%), and *iss* (12.5%) while the other VGs were identified at lower rate (Fig. 5B).

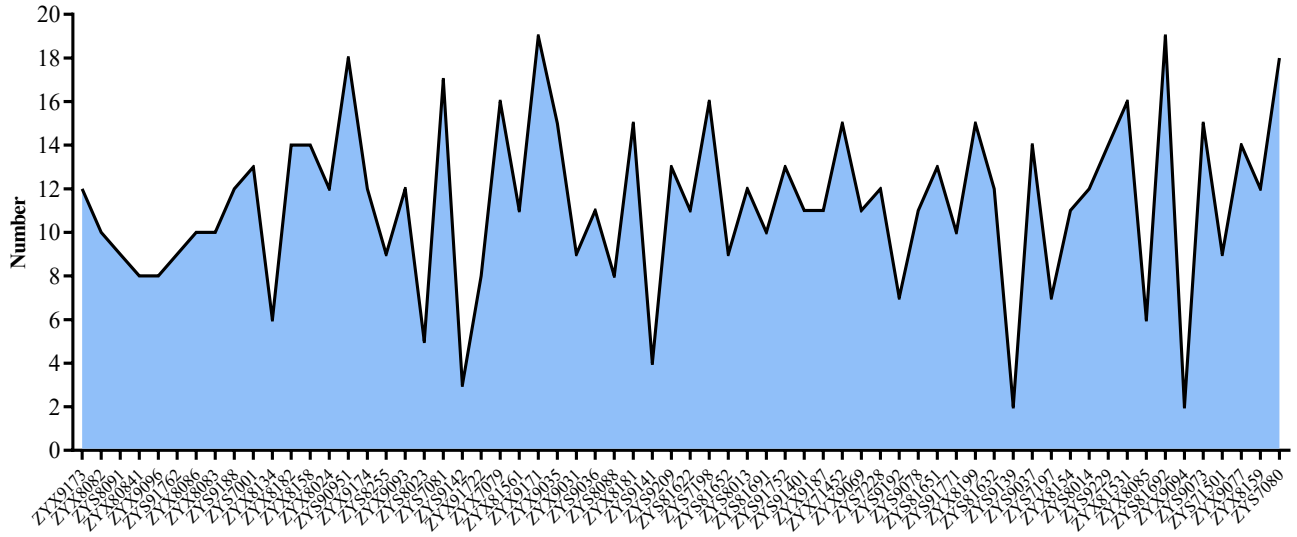

Fig. 3: Total number of prophage regions identified in each of the 64 MDR *E. coli* isolates.

Table 3: Prophage region-associated antimicrobial resistance genes (ARGs), gene family, drug class, and resistance mechanisms

ARGs	No. (%)	Gene family	Drug class/classes	Resistance mechanisms
emrE	29 (45.3)	Small multidrug resistance (SMR)	Macrolides	Antibiotic efflux
emrABR	4 (6.25)	Major facilitator superfamily (MFS)	Fluoroquinolones	Antibiotic efflux
bacA	2 (3.12)	Undecaprenyl pyrophosphate related proteins	Peptides	Antibiotic target *al*teration
cpxA	2 (3.12)	Resistance-nodulation-cell division (RND)	Aminoglycoside, aminocoumarin	Antibiotic efflux
YojI	2 (3.12)	ATP-binding cassette (ABC)	Peptides	Antibiotic efflux
CRP	1 (1.56)	Resistance-nodulation-cell division (RND)	Macrolides, fluoroquinolones, penams	Antibiotic efflux
rsmA	2 (3.12)	Resistance-nodulation-cell division (RND)	Fluoroquinolone, diaminopyrimidine, phenicol	Antibiotic efflux
mdfA	2 (3.12)	Major facilitator superfamily (MFS)	Tetracycline, disinfecting agents and antiseptics	Antibiotic efflux
tet(A)	6 (9.37)	Major facilitator superfamily (MFS)	Tetracycline	Antibiotic efflux
kdpE	1 (1.56)	kdpDE	Aminoglycoside	Antibiotic efflux
mdtABC	1 (1.56)	Resistance-nodulation-cell division (RND)	Aminocoumarin	Antibiotic efflux
mdtG	2 (3.12)	Major facilitator superfamily (MFS)	Phosphonic acid	Antibiotic efflux
mdtH	2 (3.12)	Major facilitator superfamily (MFS)	Fluoroquinolone	Antibiotic efflux
mdtM	9 (14.1)	Major facilitator superfamily (MFS)	Fluoroquinolone, lincosamide, nucleoside, phenicol, Disinfecting agents and antiseptics	Antibiotic efflux
mdtP	4 (6.25)	Major facilitator superfamily (MFS)	Nucleoside, disinfecting agents and antiseptics	Antibiotic efflux
baeR	1 (1.56)	Resistance-nodulation-cell division (RND)	Aminoglycoside, aminocoumarin	Antibiotic efflux
H-NS	4 (6.25)	Major facilitator superfamily (MFS)	Macrolide, fluoroquinolone, cephalosporin, Cephamycin, penam, tetracycline	Antibiotic efflux
marA	2 (3.12)	Resistance-nodulation-cell division (RND), General Bacterial Porin with reduced permeability to beta-lactams	Fluoroquinolone, monobactam, carbapenem, Cephalosporin, glycylcycline, cephamycin, penam, Tetracycline, rifamycin, phenicol, Disinfecting agents and antiseptics	Antibiotic efflux, Reduced permeability to antibiotic
leuO	1 (1.56)	Major facilitator superfamily (MFS)	Nucleoside, disinfecting agents and antiseptics	Antibiotic efflux
acrEF	2 (3.12)	Resistance-nodulation-cell division (RND)	Fluoroquinolone, cephalosporin, cephamycin, penam	Antibiotic efflux
acrS	2 (3.12)	Resistance-nodulation-cell division (RND)	Fluoroquinolone, cephalosporin, glycylcycline, Cephamycin, penam, tetracycline, rifamycin, phenicol, Disinfecting agents and antiseptics	Antibiotic efflux
soxS	2 (3.12)	ATP-binding cassette (ABC), Major facilitator superfamily (MFS), Resistance-nodulation-cell division (RND), General Bacterial Porin with reduced permeability to beta-lactams	Fluoroquinolone, monobactam, carbapenem, Cephalosporin, glycylcycline, cephamycin, penam, Tetracycline, rifamycin, phenicol, Disinfecting agents and antiseptics	Antibiotic target *al*teration, Antibiotic efflux, Reduced permeability to antibiotic
soxR	2 (3.12)	ATP-binding cassette (ABC), Major facilitator superfamily (MFS), Resistance-nodulation-cell division (RND)	Fluoroquinolone, cephalosporin, glycylcycline, Cephamycin, penam, tetracycline, rifamycin, phenicol, Disinfecting agents and antiseptics	Antibiotic target *al*teration, Antibiotic efflux
kpnE/F	1 (1.56)	Small multidrug resistance (SMR)	Macrolide, aminoglycoside, cephalosporin, tetracycline, Peptide, rifamycin, disinfecting agents and antiseptics	Antibiotic efflux
acrAB-tolC-marR	3 (4.69)	Resistance-nodulation-cell division (RND)	Fluoroquinolone, cephalosporin, glycylcycline, Cephamycin, penam, tetracycline, rifamycin, phenicol, Disinfecting agents and antiseptics	Antibiotic target *al*teration, Antibiotic efflux
qacE	1 (1.56)	Major facilitator superfamily (MFS)	Disinfecting agents and antiseptics	Antibiotic efflux
qacL	1 (1.56)	Small multidrug resistance (SMR)	Disinfecting agents and antiseptics	Antibiotic efflux
sitABCD	1 (1.56)	---	---	---
cmlA1	1 (1.56)	Major facilitator superfamily (MFS)	Phenicol antibiotic	Antibiotic efflux
mphA	1 (1.56)	Macrolide phosphotransferase (MPH)	Macrolide	Antibiotic inactivation
mrx	2 (3.12)	Macrolide phosphotransferase (MPH)	Macrolide	Antibiotic inactivation
parC	3 (4.69)	Fluoroquinolone resistant parC	Fluoroquinolone	Antibiotic target *al*teration
qnrS1	2 (3.12)	Quinolone resistance protein	Fluoroquinolone	Antibiotic target protection
sul1	1 (1.56)	Sulfonamide resistant sul	Sulfonamide	Antibiotic target replacement
sul2	6 (9.37)	Sulfonamide resistant sul	Sulfonamide	Antibiotic target replacement
sul3	1 (1.56)	Sulfonamide resistant sul	Sulfonamide	Antibiotic target replacement
dfrA17	1 (1.56)	Rrimethoprim resistant dihydrofolate reductase dfr	Diaminopyrimidine	Antibiotic target replacement
aadA	1 (1.56)	ANT(3")	Aminoglycoside	Antibiotic inactivation
aadA3	1 (1.56)	ANT(3")	Aminoglycoside	Antibiotic inactivation
aadA5	1 (1.56)	ANT(3")	Aminoglycoside	Antibiotic inactivation
aph(3")-Ib	5 (7.81)	APH(3")	Aminoglycoside	Antibiotic inactivation
aph(6)-Id	7 (10.9)	APH(6)	Aminoglycoside	Antibiotic inactivation
bla_{TEM-1}	1 (1.56)	TEM beta-lactamase	Monobactam, cephalosporin, penam, penem	Antibiotic inactivation
bla_{LAP-2}	1 (1.56)	LAP beta-lactamase	Cephalosporin, penam, penem	Antibiotic inactivation
bla_{CARB-2}	1 (1.56)	CARB beta-lactamase	Penam	Antibiotic inactivation
bla_{EC-13}	3 (4.69)	EC beta-lactamase	Cephalosporin	Antibiotic inactivation
bla_{EC-15}	2 (3.12)	EC beta-lactamase	Cephalosporin	Antibiotic inactivation
$bla_{CTX-M-55}$	1 (1.56)	CTX-M beta-lactamase	Cephalosporin	Antibiotic inactivation

Collectively, these findings indicate that while GIs in dairy farm waste *E. coli* are frequently involved in virulence gene dissemination, they play a more limited role in ARG carriage compared to other MGE classes such as plasmids and prophages.

Table 4: Prophage-region associated virulence genes and their function

Virulence genes	No. carrying	Percentage (%)	Function
cvaC	1	1.56	Microcin C
cma	1	1.56	Colicin M activity
iroN	1	1.56	Enterobactin siderophore receptor protein
aslA	6	9.37	Unknown
terC	2	3.12	Tellurium ion resistance protein
nlpI	2	3.12	lipoprotein Nlpl precursor
papC	4	6.25	Outer membrane usher P fimbriae
csgA	1	1.56	curlin major subunit
astA	1	1.56	Heat-stable enterotoxin EAST-1
anr	6	9.37	AraC negative regulator
yehA	3	4.69	Outer membrane lipoprotein
yehB	3	4.69	Usher protein
yehC	3	4.69	Chaperone protein
yehD	3	4.69	Major pilin subunit
fimH	12	18.7	Type 1 fimbriae
iucC	1	1.56	Aerobactin synthetase
sitA	1	1.56	Iron transport protein
iutA	1	1.56	Ferric aerobactin receptor
iss	29	45.3	Increased serum survival
traJ	2	3.12	Positive regulator of conjugal transfer operon
hlyE	2	3.12	Avian *E. coli* haemolysin
hlyF	7	10.9	Hemolysin F
etsC	6	9.37	Putative type I secretion outer membrane protein
fyuA	6	9.37	Siderophore receptor
irp2	6	9.37	High molecular weight protein 2 non-ribosomal peptide synthetase
ompT	40	62.5	Outer membrane protease (protein protease 7)
hra	2	3.12	Heat-resistant agglutinin
capU	2	3.12	Hexosyltransferase homolog

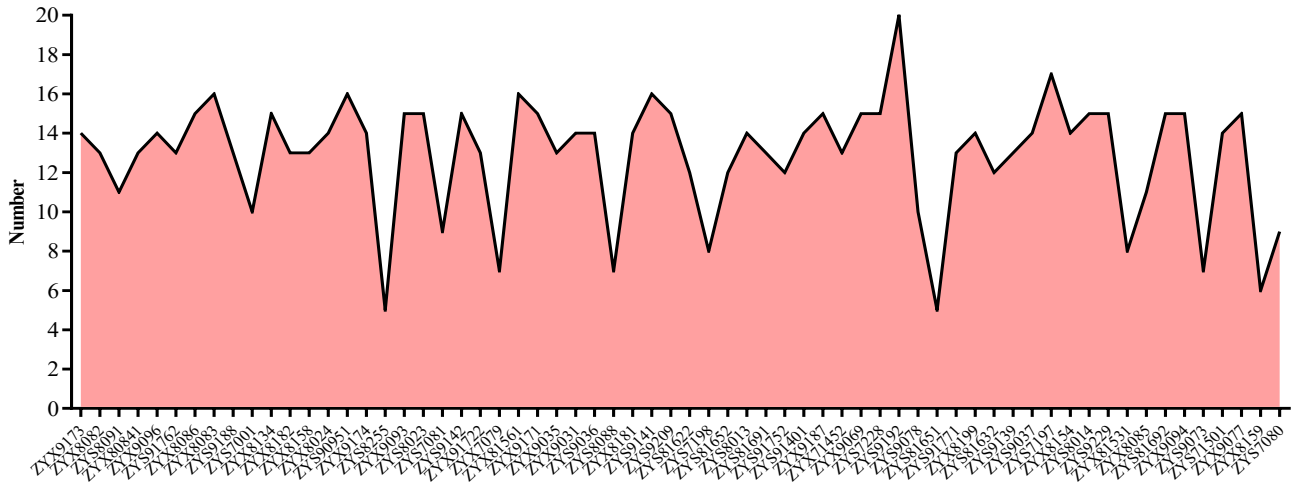

Fig. 4: Total number of genomic islands identified in each of the 64 MDR *E. coli* isolates.

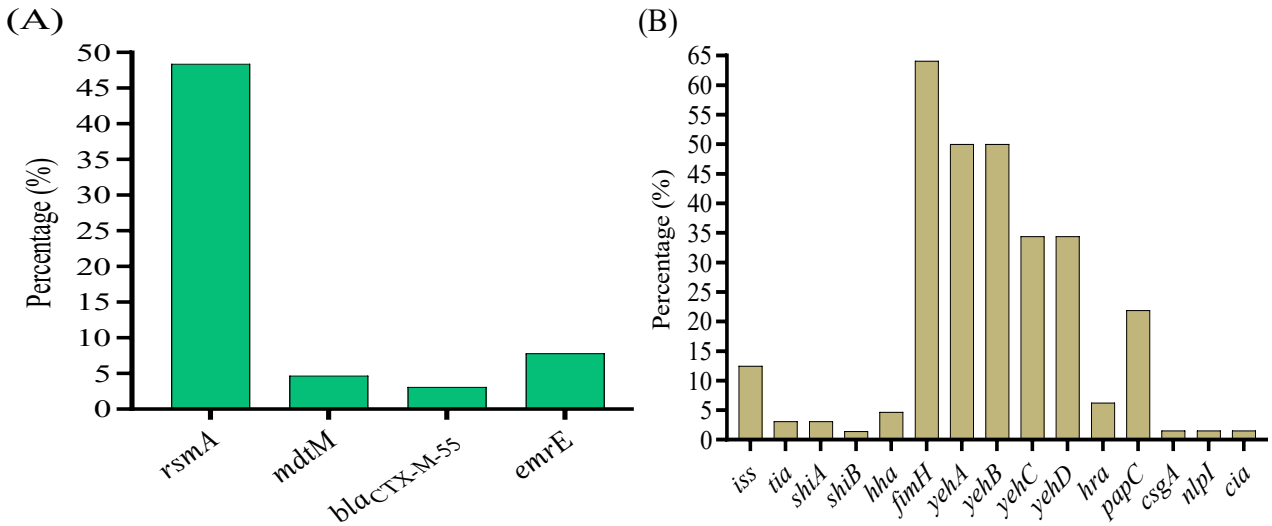

Fig. 5: (A) ARGs identified in genomic island regions of 64 MDR *E. coli* isolates. **(B)** VGs identified in genomic island regions of 64 MDR *E. coli* isolates.

DISCUSSION

Mobile genetic elements (MGEs) such as ISs and Tns are widely present in bacterial genome and impart an important role in reshaping the host genome (Shikov *et al*., 2023). These elements enable the transfer and integration of multiple genes or extensive DNA segments into diverse genomic replicons, including plasmids, viral genomes, and bacterial chromosomes (Olasz *et al*., 2022; Wu *et al*., 2024). In the present study, among the ISs, MITEEc1 belong to IS*630* family were identified as the predominant (100%, 64/64) mobile element followed by IS*Ec1* belong to ISAs1 (92.2%), IS*609* belong to IS*605* family (73.4%), IS*Vsa3* of IS*91* family (68.7%), IS*30* (67.2%), IS*5075* (56.2%), IS*Ec17* (43.7%), IS*Ec38*, and IS*Kpn8* (34.4% each). Among the transposons, the unit transposon Tn*1000* was found predominantly in 43.7% isolates while Tn*2* was only detected in single isolate. Moreover, a total of 8 different composite transposon was identified. Among the composite transposons, cn_5129_IS*Vsa3* was identified in 10.9% isolates followed by cn_1670_IS*Ec1* (7.81%), cn_4516_IS*3* and cn_12321_IS*629* (3.12%) while the other 6 composite transposons were identified in single isolates. The predominance of IS*26* in environmental *E. coli* isolates has also been noted previously (Sekizuka *et al*., 2019; Salgueiro *et al*., 2023; Wang *et al*., 2023b). The identification of IS*30* as new insertion sequence was first reported by Caspers *et al*. (1984) in a *E. coli* K12 strain and now in many environmental isolates (Zhi *et al*., 2019; Yu *et al*., 2022). Moreover, MITEEc1 acquired by all *E. coli* isolates in this study and found to be the most prevalence MGE which is in accordance with the findings of a previous study (Salgueiro *et al*., 2023). The gene carrying MGEs (also called as transposons), Tn*1000* unit transposon was identified in current study isolates has also been reported previously (Brkljacic *et al*., 2022; Syed Abu Thahir *et al*., 2023). The composite transposon facilitate the dissemination of ARGs via the mechanism of transposition (Brkljacic *et al*., 2022). In the current study, we discussed diverse MGEs and their possible role as key mediators in the horizontal transfer of ARGs needs to be explored through further studies. These transfers can occur within and between bacterial species in the environment and can ultimately reach human and animal settings via various routes.

Prophage regions frequently contribute to host survival mechanisms and assist in increasing the genetic diversity of the host. Prophage regions also act as vehicles for diverse genes to spread horizontally (Kondo *et al*., 2020). The most abundantly identified ARG was *emrE* (45.3%) confer resistance to macrolides by acting as efflux pump, followed by *mdtM* (14.1%) confer resistance to multiple antimicrobials, *aph(6)-Id* (10.9%) confer resistance to aminoglycosides by antibiotic inactivation, *sul2* and *tet(A)* (9.37% each) confer resistance to sulfonamides and tetracyclines respectively, *aph(3'')-Ib* (7.81%) confer resistance to aminoglycosides, *emrABR* complex, *mdtP*, and *H-NS* (6.25% each), *acrAB-tolC-marR*, *parC*, and *EC-13* (4.69%). Among the virulence genes, *ompT* were predominantly identified in 62.5% isolates, followed by *iss* (45.3%), *fimH* (18.7%), *hlyF* (10.9%), *aslA*, *anr*, *etsC*, *fyuA,* and *irp2* (9.37%), *yehABCD*, and *papC* (6.25%), *terC*, *nlpI*, *traJ*, *hlyE*, *hra*, and *capU* (3.12%). The role of prophage regions in harboring various ARGs has also been studied previously in different ecological niches (Marti *et al*., 2014; Kondo *et al*., 2020; Huang *et al*., 2023). Previously, novel variant of Salmonella genomic island 1 in avian *E. coli* indicates its horizontal transfer and enhances the antimicrobial resistance traits of particular pathogen (Cummins *et al*., 2019). These results highlight that prophage regions can act as potential reservoir or carriers of ARGs along with other mobile elements and play an important role in their spread through HGT.

GIs also have the ability to transfer among the bacterial species through transformation, transduction or conjugation mechanisms (Lee *et al*., 2022), but conjugation is considered as a primary mode of dissemination (Horne *et al*., 2023). Moreover, GIs can also serve as niche and procure the gene elements and disseminate through HGT (Horne *et al*., 2023). In the present study, among the GIs associated VFs, *fimH* was identified as predominant in 64.1% isolates followed by *yehAB* (50%), *yehCD* (34.4%), *papC* (21.9%), and *iss* (12.5%). Among the ARGs, *rsmA* confer resistance to fluoroquinolone, diaminopyrimidine, and phenicol were identified in 48.4% followed by *emrE* (7.81%), *mdtM* (4.69%), and $bla_{\text{CTX-M-55}}$ (3.12%). Previously, fluoroquinolone, azithromycin, rifampicin, and tetracycline etc., resistance genes have also been identified on genomic islands in *Chlamydia trachomatis* (Mestrovic and Ljubin-Sternak, 2018). Another study conducted by Dong *et al*. (2020) identified erythromycin, azithromycin (belong to macrolides), tetracycline, amikacin and gentamicin (belong to aminoglycosides), and other resistance genes on two genomic islands acquired by HGT. These findings also highlight that genomic islands can be the potential reservoir of novel ARGs and can transfer through HGT. This new perspective of dissemination of ARGs within and between the bacterial species should be considered while preparing AMR control guidelines.

The presence of these MGEs in dairy farm waste represents a critical control point for One Health interventions. From this environmental reservoir, these elements could contaminate surrounding soil and water, be taken up by wildlife or insects, and ultimately enter the food chain or human water supplies, facilitating the spread of MDR pathogens into clinical settings.

While our study provides detailed genomic insights into the mobilome of MDR *E. coli* from dairy farm waste, we acknowledge the absence of concurrent epidemiological data (farm management practices, antimicrobial usage, and environmental parameters) and short-read sequencing. Such data would enable correlation of genomic features with specific selective pressures (e.g., antimicrobial usage patterns) and environmental conditions (e.g., waste storage methods). The isolates were derived from a previous study (Shoaib *et al*., 2024) that focused on genomic characterization; therefore, detailed farm-level metadata were not available. Future studies integrating systematic collection of epidemiological metadata with long-read whole-genome sequencing will be essential to fully understand the drivers of ARG–VG convergence in livestock-associated environments.

The use of short-read assemblies limits the precise delineation of prophage boundaries, integration sites, and the distinction between intact, defective, or extrachromosomal prophage regions. While PHASTEST provides high-confidence predictions (score ≥90), these remain *in-silico* inferences that require experimental validation or long-read sequencing for complete structural resolution. Therefore, our findings should be interpreted as ARGs and VGs residing within genomic regions predicted to be prophage-derived, rather than definitive evidence of prophage-mediated mobilization.

Conclusions: In this study, we identified 64 multidrug resistant *E. coli* isolates carrying diverse ARGs and VGs. The bioinformatics analysis revealed that *sul2*, *rmtB*, *aph(3")-Ib*, *aph(6)-Id*, $bla_{\text{TEM-1}}$, *floR*, and *tet(A)* genes were predicted to be predominantly associated with plasmids. For VGs, *traT*, *traJ*, *anr*, *iutA*, and *iucC* genes were predominately predicted in plasmid contigs. Moreover, the unit transposon and composite transposon were also found to carry ARGs (e.g., $bla_{\text{TEM-1}}$, *floR*) and VGs (e.g., *clpK*). Similarly, prophage regions and genomic island regions were also carrying high percentage of ARGs and VGs in *E. coli*. The co-occurrence of ARGs and VGs on diverse mobilomes in *E. coli* may indicate their high potential of dissemination within and between the bacterial pathogens of animal and human significance. Furthermore, our findings highlight the urgent need for application of integrated One Health policies to mitigate the potential risks at animal-human-environment interface.

Competing interests: The authors declare no competing interests.

Data availability: The sequencing data belong to this study has been submitted in the GenBank under the BioProject ID: PRJNA1117893.

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