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Genomic Characteristics of an Extensive-Drug-Resistant Clinical *Escherichia coli* O99 H30 ST38 Recovered from Wound

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Abstract

Background: Antibiotic-resistant *Escherichia coli* is one of the major opportunistic pathogens that cause hospital-acquired infections worldwide. These infections include catheter-associated urinary tract infections (UTIs), ventilator-associated pneumonia, surgical wound infections, and bacteraemia.

Objectives: To understand the mechanisms of resistance and prevent its spread, we studied *E. coli* C91 (ST38), a clinical outbreak strain that was extensively drug-resistant. The strain was isolated from an intensive care unit (ICU) in one of Kuwait's largest hospitals from a patient with UTI.

Methods: This study used whole-genome sequencing (Illumina, MiSeq) to identify the strain's multi-locus sequence type, resistance genes (ResFinder), and virulence factors. This study also measured the minimum inhibitory concentrations (MIC) of a panel of antibiotics against this isolate.

Results: The analysis showed that *E. coli* C-91 was identified as O99 H30 ST38 and was resistant to all antibiotics tested, including colistin (MIC > 32 mg/L). It also showed intermediate resistance to imipenem and meropenem (MIC = 8 mg/L). Genome analysis revealed various acquired resistance genes, including *mcr-1*, *bla_{CTX-M14}*, *bla_{CTX-M15}*, and *bla_{OXA1}*. However, we did not detect *bla_{NDM}* or *bla_{VIM}*. There were also several point mutations resulting in amino acid changes in chromosomal genes: *gyrA*, *parC*, *pmrB*, and *ampC* promoter. Additionally, we detected several multidrug efflux pumps, including the multidrug efflux pump *mdf(A)*. Eleven prophage regions were identified, and PHAGE_Enterо_Sfl_NC was detected to contain ISEc46 and ethidium multidrug resistance protein E (*emrE*), a small multidrug resistance (SMR) protein family. Finally, there was an abundance of virulence factors in this isolate, including fimbriae, biofilm, and capsule formation genes.

Conclusions: This isolate has a diverse portfolio of antimicrobial resistance and virulence genes and belongs to ST38 O99 H30, posing a serious challenge to treating infected patients in clinical settings.

Keywords: Whole Genome Sequencing, Colistin Resistance, Virulence Factors, Antimicrobial Resistance, Insertion Sequences

1. Background

Multi-drug resistant *Escherichia coli* are opportunistic pathogens causing hospital-acquired infections worldwide. These infections include catheter-associated urinary tract infections, ventilator-associated pneumonia, surgical wound infections, and bacteraemia. They often carry resistance genes to antibiotics, such as β -lactams and fluoroquinolone, that are commonly used for treatment. Genes encoding extended-spectrum β -lactamases (ESBLs) are often found on mobile genetic elements (MGEs) and are harbored within transposons or insertion sequences, thereby facilitating their spread

to other strains. The most prevalent and dominant ESBL gene found in *Enterobacteriaceae* isolated from humans and food-producing animals is *bla_{CTX-M15}* (1). Recently, a major concern has been the resistance to colistin, a polymixin, one of the last antibiotics in use after others failed. Colistin resistance gene *mcr*, which currently has ten variants, is usually found on plasmids of various incompatibility groups (IncX4, IncI2, and IncHI2) and often coexists with ESBLs (2, 3). In addition to ESBL genes, macrolide, tetracycline, aminoglycoside, fluoroquinolone, and carbapenem resistance genes can also coexist in a colistin-resistant isolate, limiting treatment options for

hospitalized patients.

Plasmid (*mcr*-) and chromosomal-mediated colistin resistance involve mutations in genes encoding enzymes that are associated with outer membrane modification of LPS by encoding a phosphoethanolamine transferase that catalyzes the addition of a phosphoethanolamine moiety to lipid A (3, 4), such as the *pmrC* and *pmrE* and the *pmrHFIJKLM* operon (4). Previous studies on *E. coli* have revealed that mutations in the sensor histidine kinase *pmrB* are an important mechanism of colistin resistance, leading to the constitutive production of the enzymes ArnT and EptA that add a positive charge (4-amino-4-deoxy-L-arabinose and phosphoethanolamine, respectively) to the phosphate groups of lipid A and reducing the affinity of colistin to bind to lipid A (3, 4).

To plan effective treatment guidelines, it is crucial to understand the mechanisms of resistance and epidemiology of multidrug-resistant (MDR) *E. coli* in both the community and hospitals. Given the burden of diseases caused by *E. coli* and its significant public health concern, hospitals should continuously monitor their antimicrobial treatment efficacy. Whole-genome sequencing (WGS)-based in silico approaches are valuable tools in gene analysis of outbreak strains that offer detailed epidemiological investigation and tracing of pathogens (5). In this study, we used WGS to characterize *E. coli* C91 (ST38), an extensively drug-resistant clinical outbreak strain isolated from patient zero in the intensive care unit (ICU) of one of the largest hospitals in Kuwait, with the intention of successfully treating the patients and containing its spread.

2. Methods

2.1. Sample Collection

A clinical *E. coli* isolate C91 was isolated from a post-surgical wound of a 53-year-old male admitted to ward 8/ICU (26/11/2016) and was initially identified by VITEK 2 ID system (bioMérieux, Marcy-l'Etoile, France). This patient was named patient zero.

2.2. Antibiotic Sensitivity Testing

Antimicrobial sensitivity testing was carried out according to the Clinical and Laboratory Standards Institute (2020) (6). The minimum inhibitory concentrations (MICs) were determined for aminoglycosides, chloramphenicol, tetracycline, β -lactams, including carbapenems, and in combination with β -lactam inhibitors, ciprofloxacin, erythromycin, trimethoprim, gentamycin, and colistin. The MIC ($\mu\text{g}/\text{mL}$) against a panel of antibiotics were determined using E test

(bioMérieux, Marcy-l'Etoile, France). For colistin, the agar dilution method was used (6).

2.3. Whole-Genome Sequencing Analyses

Genomic deoxyribonucleic acid (DNA) was extracted using QIAamp[®] DNA Mini Kit (Qiagen, Hilden, Germany) and quantified by the NanoDrop-800 spectrophotometer (Thermo Fisher Scientific, Wilmington, NC, USA) according to the manufacturer's instructions. The WGS was performed by MicrobesNG, University of Birmingham, UK (<https://microbesng.uk>) using the Illumina MiSeq[®] sequencer platform. The reads were trimmed using Trimmomatic, and the quality was assessed by MicrobesNG's in-house scripts combined with the following software packages: SAMtools (Sequence, Alignment/Map), Bedtools, and bwa-mem (Burrows-Wheeler Aligner). All statistics are based on contigs of size ≥ 500 bp unless otherwise noted. The trimmed data were assembled using the SPAdes algorithm assembler (version: 3.7.1); this de novo assembly of the quality-controlled reads was assembled to create a draft genome sequence, and variant calling was performed using VarScan. An automated annotation was performed using Prokka (version 1.13.3). The WGS of the isolate was submitted to Genbank Accession: SAMN10105215, ID: 10105215 (sample name: *Escherichia coli* strain Kuwait C-91).

2.4. In Silico Molecular Analysis

For in silico WGS analysis, the assembled sequences were uploaded onto the Center for Genomic Epidemiology to identify the following: ResFinder v4.3.3, ResFinderFG 2.0, KmerResistance 2.2 (7), PathogenFinder1.1 (8), VirulenceFinder 2.0 (9-11), multilocus sequence typing 2.0 (12), PlasmidFinder 2.1 (13), MGE v1.0.3 (14), SerotypeFinder 2.0 (15), FimTyper 1.0 (16), and 2.1 (13), CHTyper 1.0 (17), CARD 2020 annotation (18), Pfam (InterPro 95.0), VirSorter2 version 2.2.4 (<https://u.osu.edu/viruslab/>). The presence of insertion sequences was confirmed using ISFinder (19). Proksee CGView.js server was used for genome assembly, annotation, and visualization and provided a complete genome CGView/Proksee map JSON file (20).

2.5. Detection of Phages from WGS

Phaster tool (21) was used to identify prophage sequences. This tool classifies the phages into three classes (intact, questionable, and incomplete) based on their completeness (phage score). Additionally, by using the Proksee server, phages were identified with the VirSorter2 2.2.4 tool and were screened for antimicrobial resistance genes using the basic local alignment search tool (BLAST).

3. Results

3.1. Description of the Isolate and Mapping Summary

The bacterial strain C91 was identified as *E. coli* O99 H30 ST38 according to two different schemes, Warwick and Pasteur Institute (Appendix 1). The draft genome was annotated using RAST (Table 1) and revealed a linear chromosome consisting of 5 532 235 base pairs, with 4 964 coding sequences, 87 transfer RNA (tRNA) genes, and several proteins with functional assignments. The genome was assembled using the SPAdes assembler (version: 3.7.1) from trimmed data, producing an N50 quality value of 181 117 and a L50 of 11, with an N75 of 97 722 and L75 of 20. The sample had a mapping rate of 76.81% against the reference genome (without Ns), with an average depth of 76.96X and over 90.93% coverage of more than 1X, a result that falls within the normal range. The genome mapping of antimicrobial resistance and virulence factors is shown in Figure 1, and the comparison of *E. coli* C91 to *E. coli* K12 MG1655 (GenBank: U00096.2) using NCBI and Proksee software is presented in Figure 2.

Table 1. Summary of the Statistics of the Assembled Genome of *E. coli* C91

<i>E. coli</i> C91	Values
Genome size (bp)	5 532 235
Total length of the genes (bp)	4 529 853
GC content %	51.45
Number of genes	4 964
% of genome (genes)	86.67
Gene average length (bp)	913
Gene internal length	696 465
Gene internal GC content	43.87
% of genome (internal)	13.33
Average depth	76.96X
Contigs	183
Largest contig	399 443
Genome coverage	90.93%
GC%	50.42
N50	181 117
N75	97 722
N90	66 020
L50	11
L75	20
sRNAs	78
tRNAs	87

3.2. Plasmids and MGEs

Five plasmids IncY, IncI2(Delta), IncFIC(FII), IncI1-I(Alpha), and IncFIBIncF and 17 MGEs, including Tn7, were detected harboring antibiotic resistance genes. Their locations are shown in Table 2.

3.3. Antibiotic Sensitivity Testing and Resistance Genes

E. coli C91 was resistant to all antibiotics tested, including aminoglycosides, chloramphenicol, tetracycline, β -lactams (both alone and in combination with β -lactam inhibitors), ciprofloxacin, erythromycin, trimethoprim, gentamycin, and colistin. The MIC for these antibiotics was greater than 32 mg/L. It also demonstrated intermediate resistance to imipenem and meropenem with an MIC of 4 mg/L. The analysis of the genome of *E. coli* C91 revealed the presence of 200 antibiotic-resistance genes, including efflux pump complexes and antibiotic target protection proteins, as confirmed by CARD annotations. The genome analysis also revealed the presence of *mcr-1*, *bla*_{CTX-M-14}, *bla*_{CTX-M-15}, and *bla*_{OXA-1} genes, but not *bla*_{NDM} or *bla*_{VIM}. The bacterium was observed to have acquired resistance genes, including *aac(3)-IIa*, *aac(6')Ib-cr*, *aadA1*, *qnrS1*, *catB4*, *tetA*, *mphA*, *ermB*, and *dfrA1*, as shown in Table 3 (and Appendix 2) and Figure 1. Point mutations were also detected in chromosomal resistance genes, including *gyrA*, *parC*, and *pmrB*, leading to changes in amino acids, as shown in Table 4. The results of some of these mutational modifications are not clear.

3.4. Virulence Factors

This isolate has an abundance of virulence factors shown in Table 5 and Figure 1, including fimbriae, biofilm, and capsule formation genes.

3.5. Phage Analysis

Eleven prophage regions were identified in *E. coli* C91, from contig 1 - 45, using the Phaster tool (Table 6 Appendix 3). Out of these regions, three are intact, seven are incomplete, and one is questionable. However, when the VirSorter2 2.2.4 tool was used in Proksee software, phages were also picked up from nodes 46-183 (Table 6). One of the intact phages is PHAGE_Salmon_SJ46_NC_031129(89)(IncY), located on NODE_22_length_94041_cov_7.78437. On NODE_12, PHAGE_Enterococcus_Sfl_NC_027339(6) (partial sequence) was detected, containing ISEc46 and *emrE* (ethidium multidrug resistance protein E), an SMR protein family.

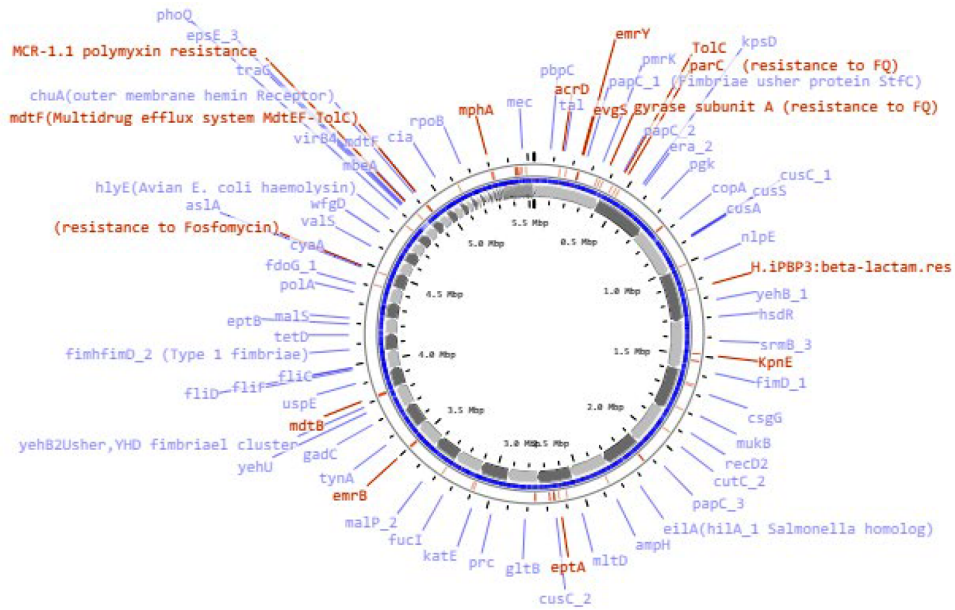


Figure 1. The gene map of *E. coli* C91 with labels showing the resistance (red) and virulence (blue) genes.

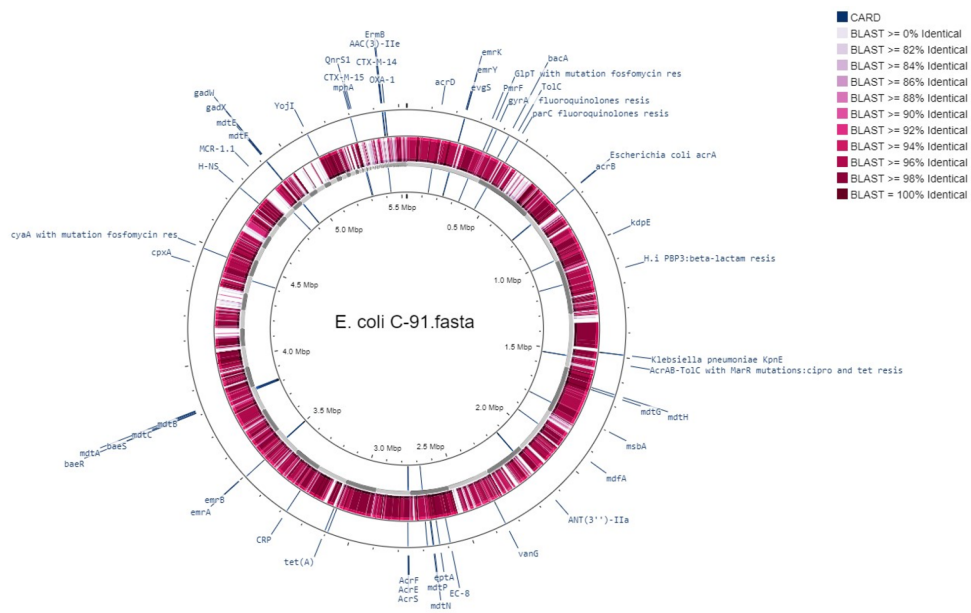


Figure 2. Color existing basic local alignment search tool (BLAST) features by percent identity and sort BLAST tracks by similarity, *E. coli* C91 backbone vs. *E. coli* K12 MG1655 (GenBank: U00096.2).

Table 2. Plasmids Identified in *E. coli* C91

Plasmid	Contig	Position in Contig	Coverage %	Identity %	Accession No.	Resistance Genes/Phage
IncY	NODE_22_length_94041_cov_7.78437	24344..25108	100	99.74	K02380	Circular phage/ PHAGE_Salmon_SJ46_NC.031129(89)
IncI2(Delta)	NODE_29_length_60972_cov_14.190	4841..5156	100	98.42	AP002527	mcr-1.1
IncFIC(FII)	NODE_32_length_48168_cov_7.4304	2747..3243	99.4	94	AP001918	-
IncHI-1(Alpha)	NODE_33_length_45415_cov_9.8770	15412..15553	100	99.3	AP005147	-
IncFIB	NODE_53_length_5159_cov_9.67925	2548..3229	100	97.65	AP001918; CP053724	-

Table 4. Chromosomal Point Mutations and Their Phenotypic Characteristics Identified in *E. coli* C-91

Mutation	Nucleotide Change	Amino Acid Change	PMID	Notes
gyrA p.S83L	TCG → TTG	S → L	8891148, 2168148, 12654733, 12654733	
gyrA p.D87N	GAC → AAC	D → N	12654733, 12654733, 12654733, 22878251, 12654733, 1850972	D87G or D87Y confer resistance to nalidixic acid only, if occurring alone. Unknown phenotype if D87H occurs alone
gyrA p.D678E	GAC → GAA	D → E	Phenotype not found in database	Unknown phenotype
parE p.S458A	TCG → GCG	S → A	14506034, 28598203	Unknown phenotype if S458T or S458A occurs alone. Nalidixic acid and ciprofloxacin resistance when associated with <i>gyrA</i> mutations
parC p.S57T	AGC → ACC	S → T	14510643	Unknown phenotype if S57T occurs alone. Nalidixic acid and ciprofloxacin resistance when associated with <i>gyrA</i>
parC p.S80I	AGC → ATC	S → I	8851598, 8851598, 21856834-20638608, 8524852, 25631675, 25631675, 25631675	Unknown phenotype if each mutation occurs alone. Nalidixic acid and ciprofloxacin resistance when associated with <i>gyrA</i> mutations
parC p.E62K	GAA → AAG	E → K	Phenotype not found in database	Unknown phenotype
parC p.D475E	GAT → GAA	D → E	Phenotype not found in database	Unknown phenotype
parC p.K200N	AAA → AAT	K → N	Phenotype not found in database	Unknown phenotype
parC p.L344R	CTG → CGG	L → R	Phenotype not found in database	Unknown phenotype
parC p.D197E	GAC → GAG	D → E	Phenotype not found in database	Unknown phenotype
parC p.D309E	GAT → GAG	D → E	Phenotype not found in database	Unknown phenotype
ampC promoter: p.R24	CGA → TGA	R → *	Phenotype not found in database	Unknown phenotype
pmrB: p.H2R	CAT → CGT	H → R	Phenotype not found in database	Unknown phenotype
pmrB: p.D283G	GAC → GGC	D → G	Phenotype not found in database	Unknown phenotype
pmrB: p.Y315F	TAT → TTT	Y → F	Phenotype not found in database	Unknown phenotype

Table 6. Phage Analysis with Phaster Tool Indicative of the Regions Containing Phages ^a

	Region	Region Length (kb)	Completeness	# Total Proteins	Most Common Phage	GC %
NODE_5_length_285039_cov_26.0736	1	44.1	Intact	54	PHAGE_Enterо_P88_NC.026014(33)	52.82
	2	16.3	Questionable	24	PHAGE_Salmon_118970_sal3_NC.031940(50.68
NODE_12_length_170122_cov_27.8913	3	26.8	Incomplete	24	PHAGE_Enterо_sfl_NC.027339(6)	45.39
NODE_18_length_114548_cov_26.6919	4	26.9	Incomplete	21	PHAGE_Shigel_POCl3_NC.025434(6)	45.95
NODE_19_length_110482_cov_29.551	5	27.8	Incomplete	31	PHAGE_Enterо_phiP27_NC.003356(13)	48.50
NODE_22_length_94041_cov_7.78437	6	92.5	Intact	117	PHAGE_Salmon_SJ46_NC.031129(89)	48.07
NODE_34_length_38724_cov_8.49753	7	9.1	Incomplete	14	PHAGE_Rhodoc_RGL3_NC.016650(1)	56.85
NODE_39_length_24718_cov_29.6434	8	24.3	Intact	28	PHAGE_Pseudo_phiPSA1_NC.024365(7)	48.89
NODE_40_length_20447_cov_25.9281	9	19.9	Incomplete	20	PHAGE_Enterо_lambda_NC.001416(19)	56.34
NODE_43_length_11726_cov_19.4543	10	8.9	Incomplete	11	PHAGE_Microc_MaMV_DC_NC.029002(2)	51.76
NODE_45_length_8140_cov_11.3055	11	7.6	Incomplete	9	PHAGE_Escher_RCS47_NC.042128(3)	48.13

^a Region: The number assigned to the region. Region length: The length of the sequence of that region (in bp). Completeness: A prediction of whether the region contains an intact or incomplete prophage. # Total proteins: The number of ORFs present in the region. Most common phage: The phage(s) with the highest number of proteins most similar to those in the region. GC %: The percentage of GC nucleotides of the region.

Table 5. Virulence Factors, Protein Function, and Their Position in Contig

Virulence Factor	Identity	Query/Template Length	Contig	Position in Contig	Protein Function	Accession Number
<i>AsfA</i>	98.31	1656/1656	NODE_24_length_92227.cov.34.925	37443..39098	Contributing to the invasion of brain microvascular endothelial cells	CP022686
<i>αamR:FN554766</i>	99.84	645/645	NODE_2_length_314467.cov.36.7095	209279..209923	Not known	
<i>Air</i>	95.16	4604/4605	NODE_8_length_222074.cov.39.2814	120721..125324	Enterococcal aggregative immunoglobulin repeat protein	CP003034
<i>Air</i>	96.24	213/213	NODE_32_length_48168.cov.7.4304	4169..4381	AraC negative regulator	AL391753
<i>capU</i>	99.91	1089/1089	NODE_38_length_25756.cov.32.6998	7151..8239	Hexosyltransferase homolog	CU928145
<i>chuA</i>	100	1983/1983	NODE_30_length_56813.cov.40.9025	37851..39833	Outer membrane hemin receptor	UFZU01000002
<i>Cia</i>	100	147/147	NODE_33_length_45415.cov.9.87703	8729..8875	Colicin	QMG801000002
<i>csgA</i>	92.98	456/456	NODE_6_length_255487.cov.30.7367	82846..83301	curlin major subunit CsgA (biofilm)	CP069646
<i>eilA</i>	98.65	1698/1698	NODE_8_length_222074.cov.39.2814	131902..133599	Salmonella HliA homolog	FN554766
<i>espY2:000868321</i>	94.56	570/570	NODE_4_length_294518.cov.34.7303	145342..145911	Not known	
<i>flicC</i>	92.15	4214/4254	NODE_9_length_221455.cov.34.9789	120658..124871	intimin-like adhesin FdeC	AP010953
<i>fimH</i>	100	489/489	NODE_20_length_97722.cov.41.3206	15817..16305	Type 1 fimbriae	NA
<i>Gad</i>	99.1	1116/1401	NODE_96_length_1120.cov.51.0514	1..1116	Glutamate decarboxylase	FN554766
<i>hlyE</i>	98.91	918/918	NODE_27_length_69431.cov.30.2889	62576..63493	Avian <i>E. coli</i> haemolysin	ECU57430
<i>Hra</i>	95.01	741/741	NODE_20_length_97722.cov.41.3206	95294..96034	Heat-resistant agglutinin	CP040456
<i>Hra</i>	100	792/792	NODE_2_length_314467.cov.36.7095	219779..220570	Heat-resistant agglutinin	CP043942
<i>Iss</i>	100	294/294	NODE_40_length_20447.cov.25.9281	19924..20217	Increased serum survival	CP001846
<i>kpsE</i>	100	1149/1149	NODE_2_length_314467.cov.36.7095	155149..156297	Capsule polysaccharide export inner-membrane protein	AAAMK02000004
<i>kpsM11.K5</i>	100	777/777	NODE_2_length_314467.cov.36.7095	141362..142138	Polysialic acid transport protein; Group 2 capsule	MG739441
<i>neuC</i>	100	1176/1176	NODE_2_length_314467.cov.36.7095	145757..146932	Polysialic acid capsule biosynthesis protein	J11W01000144
<i>nlpI</i>	99.77	885/885	NODE_11_length_18117.cov.35.2668	107595..108479	lipoprotein NlpI precursor	CP000243
<i>sifA</i>	100	915/915	NODE_16_length_138200.cov.28.3328	3778..4692	Iron transport protein	HG977190
<i>terC</i>	98.46	714/714	NODE_13_length_161358.cov.37.5202	84643..85356	Tellurium ion resistance protein	CP000468
<i>terC</i>	98.54	959/966	NODE_11_length_18117.cov.35.2668	173664..174622	Tellurium ion resistance protein	MG591698
<i>traJ</i>	98.55	690/690	NODE_32_length_48168.cov.7.4304	34241..34930	Protein TraJ (positive regulator of conjugal transfer operon)	AF550679
<i>traT</i>	100	777/777	NODE_32_length_48168.cov.7.4304	13597..14373	Outer membrane protein complement resistance	AAJW020000025
<i>yehA</i>	95.85	1035/1035	NODE_17_length_131052.cov.29.4018	90990..92024	Outer membrane lipoprotein, YHD fimbriae cluster	CP042934
<i>yehB</i>	97.5	2481/2481	NODE_17_length_131052.cov.29.4018	88494..90974	Usher, YHD fimbriae cluster	CP042934
<i>yehC</i>	96.3	675/675	NODE_17_length_131052.cov.29.4018	87804..88478	Chaperone, YHD fimbriae cluster	CP042934
<i>yehD</i>	97.24	543/543	NODE_17_length_131052.cov.29.4018	87181..87723	Major pilin subunit, YHD fimbriae cluster	CP042934

4. Discussion

Colistin-resistant *E. coli* is one of the most important nosocomial pathogens with limited treatment options. The present study characterized a multi-drug resistant clinical *E. coli* (C-91) isolate causing complications in the ICU of one of the largest hospitals in Kuwait. This isolate has a diverse collection of genes conferring resistance to an array of antimicrobial agents. It contains *bla*_{CTX-M-15}, the most dominant ESBL (22), and *bla*_{CTX-M-14} (23), in addition to other important resistance genes, including *aadA1*, *aac(3)-IIa*, *aac(6')-Ib-cr*, *bla*_{OXA-1}, *mcr-1.1*, *mph(A)*, *erm(B)*, *catB3*, *qnrS1*, *tet(A)*, *dfrA1*, and *mphA* (the most common azithromycin resistance gene detected in *E. coli*). It encodes for resistance enzyme MPH(2')-I, which inactivates 14-membered macrolides (e.g., erythromycin, telithromycin, roxithromycin) over 16-membered macrolides (e.g., tylosin and spiramycin) (24). In this study, *aac(3)-IIa*, *qnrS1*, *mph(A)*, and *bla*_{CTX-M-15} genes were associated with insertion sequence (IS) ISKpn19. *bla*_{CTX-M-14} was associated with IS102, *tet(A)*, *terC* with Tn5403, and *ant(3'')-Ia*, (*aadA1*), *dfrA1* with Tn7. In total, we identified 14 insertion sequences and transposons (Appendix 4). Insertion sequence elements can play an integral role in the transfer of these resistance genes and virulence factors in their surrounding regions (25).

Escherichia coli C91 also contains several multidrug efflux pumps, including the multidrug efflux pump *mdf(A)*, which confers resistance to antibiotics, such as chloramphenicol, erythromycin, and fluoroquinolones (26). The present study also detected mutations in chromosomally encoded *gyrA*, *gyrB*, *parC*, *pmrB*, *ampC*, and *cya* genes causing resistance to fluoroquinolones, polymyxins, and fosfomycin. We did not detect *bla*_{NDM}, *bla*_{VIM}, nor *bla*_{OXA-48} in this isolate, although the MIC for imipenem was just below the cutoff point (MIC = 4). However, others have reported the prevalence of carbapenem resistance among *Enterobacteriaceae* in hospitals in Kuwait (27).

Antimicrobial resistance plasmids present in *E. coli* C91 comprise epidemic resistance plasmids IncFIB and IncFIC(FII), which can acquire resistance determinants and disseminate readily among *Enterobacteriaceae* and broad-range IncY, IncI2 (pMCR-1) carrying the *mcr-1* gene. IncI-I plasmids have been shown to propagate the resistance genes between different species (28). Therefore, this isolate has the potential to tolerate and resist conventional antibiotic therapies.

The identification of *E. coli* clones in the fields of taxonomy and epidemiology is predicated on a combination of O- and H- antigens. These antigens are characterized by variations in the sugars present

in the O unit and the linkages between O units (29). There are currently 185 O antigens, and the O99 antigen consists of four d-rhamnose moieties in the backbone and two d-glucose moieties in the side chain. The O-antigen is synthesized and transported by an ABC transporter-dependent process and is considered an important virulence factor, offering selective advantages in specific niches. Pathogenic clones are often found to have a higher incidence of certain O antigens (29, 30).

H-antigens (flagellins) are encoded by *fliC* genes, with 53 different serotypes of H-antigen identified (31). The diversity of H-antigens arises from lateral gene transfer and recombination of foreign DNA, generating alleles and antigenic variation (32). *FimH* genes encode a type I fimbria that enables adherence and infects the epithelial urinary tract tissue expressed in uropathogenic *E. coli* (UPEC). *FliC* genes encode proteins that promote successful host colonization and are involved in interleukin-6 (IL-6) and interleukin-8 (IL-8) release. *FumC* genes encode a protein that catalyzes fumarate oxidation to malate during the oxidative TCA cycle under aerobic conditions. *FumC* is required for *E. coli* fitness in vivo, and a loss of *FumC* results in delayed growth during iron limitation (33-35).

The H30 subclone has been reported to be responsible for the clonal dissemination of ST131 *E. coli* (36). Therefore, it is proposed that H30 provides ST38 clones with the advantage of propagation. Since *E. coli* sequence type ST38 has become prominently associated with hospital- and community-acquired infections worldwide (37-39), it is crucial to identify the subclones to increase the chances of successful treatments.

In conclusion, *E. coli* C91 (ST38) O99 H30 is a high-risk and globally disseminated extraintestinal pathogenic (ExPEC) strain that can cause invasive infections and resist multiple antibiotic treatments. This study used WGS and in silico analysis to identify the molecular characteristics of this isolate. The obtained results showed that it contains genes encoding ESBLs that confer resistance to cephalosporins and other β -lactam antibiotics. Additionally, *E. coli* C91 (ST38) is resistant to macrolides, tetracyclines, aminoglycosides, and fluoroquinolones, making it extensively drug-resistant (XDR). Furthermore, it carries *mcr-1* gene, which severely limits the treatment options. This isolate also encodes several virulence factors facilitating biofilm formation and adherence to tissues. Infections caused by XDR *E. coli* C91 (ST38) O99 H30 in the ICU might be life-threatening and require urgent treatment.

Supplementary Material

Supplementary material(s) is available [here](#) [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors' Contribution: Study concept and design: Ali A Dashti and Leila Vali; analysis and interpretation of the data: Ali Dashti and Leila Vali; drafting of the manuscript: Ali A Dashti and Leila Vali; critical revision of the manuscript for important intellectual content: Ali A Dashti, Leila Vali, Sara Shamsah, and Mehrez Jadaon and Sherief ElShazly.

Conflict of Interests: The authors declare that there is no conflict of interest.

Data Availability: All data are available in publicly accessible databases under the accession numbers reported.

Ethical Approval: The authors would like to declare that the experiments performed and completed in our laboratories did not involve any human subjects, human material, or human data. Our laboratory received only the bacterial isolate on an agar culture plate without any patient number, name, or identification of any nature from the hospital laboratories. The authors were only provided with the source of sampling, age, gender, and the ward to which the patient was admitted. The authors were never in direct contact with any biological samples or patients in any way. Therefore, ethical approval and consent were not required for this study.

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Table 3. Antimicrobial Resistance Genes and Their Phenotypic Characteristics Identified in *E. coli* C91

Gene	Phenotype	Position in Contig/MCF, Plasmid	Coverage %	Identity	Accession
<i>D-alanine-D-alanine ligase van_Ilgase</i>	Cycloserine	NODE.4_length_294518_cov.34.7303.119741.118821	100	99.02	KF628564.1
<i>D-alanyl-D-alanine carboxypeptidase, none enzyme β_lactamresistance</i>	Penicillin	NODE.7_length_249411_cov.31.6866.90740.91963	99	99.75	BDB50754.1
<i>ant(3'')-Ia, (aadA1)</i>	Spectinomycin, streptomycin	NODE.8_length_222074_cov.39.2814.40651.39863/In7	100	100	JQ480156
<i>dfrA1</i>	Trimethoprim	NODE.8_length_222074_cov.39.2814.41801.41328/In	100	100	X00926
<i>Multidrug resistance protein in MdtL</i>		NODE.8_length_222074_cov.39.2814.62182.61007	100	100	WP_0000086009.1
<i>Multidrug efflux MFS transporter EmrD</i>		NODE.8_length_222074_cov.39.2814.102857.101673	100	99	WP_097336506.1
<i>van_Ilgase</i>	D-cycloserine	NODE.9_length_221455_cov.34.9789.34311.35405	100	99.02	KF628791.1
<i>β-lactamase</i>	Piperacillin	NODE.10_length_210474_cov.36.1065.48903.50061	100	99.83	KU607300.1
<i>emrE (SMR protein family)</i>	Ethidium multidrug resistance	NODE.12_length_170122_cov.27.8913/ISE46			
<i>tet(A)</i>	Tetracycline, oxytetracycline, doxycycline; minocycline	NODE.13_length_16358_cov.37.5202.2716.3915/In5	100;100	100;99.85	AJ517790;JX009293.1;GQ343144.1
<i>stLABCD</i>	Hydrogen peroxide	NODE.16_length_138200_cov.28.3328.4692.1243	99.59	97.48	AV598030
<i>Multidrug efflux system MdtABC-TolC</i>		NODE.17_length_131052_cov.29.4018.123533.115966	100	100	CP128875.1
<i>mcr-1.1</i>	Polymyxin, colistin	NODE.29_length_60972_cov.14.1908.47111.45486/Incl2(Delta)	100	100	KP347127;OMI79755.1
<i>qnrS1</i>	Ciprofloxacin	NODE.43_length_11726_cov.19.4543.6035.5379/ISKq	100	100	AB187515
<i>mph(A) (Macrolide phosphotransferase)</i>	Azithromycin, telithromycin, erythromycin, spiramycin	NODE.43_length_11726_cov.19.4543.197.1102/ISKpn19	100	100	D16251
<i>blaCTX-M-15 (Class A)</i>	Ticarcillin, aztreonam, ampicillin, amoxicillin, piperacillin, ceftazidime, cefotaxime, ceftriaxone, ceftipime	NODE.43_length_11726_cov.19.4543.11551.10676/ISK	100	100	AY044436;GQ343005.1
<i>blaCTX-M-44; (Class A, blaCTX-M-44a IIIc)</i>	Ticarcillin, aztreonam, ampicillin, amoxicillin, piperacillin, ceftazidime, cefotaxime, ceftriaxone, ceftipime	NODE.65_length_3010_cov.7.26882.2841.1966/IS102	100;100	100;99.89	AF252622;KU544013.1
<i>aminoglycoside N(3')-acetyltransferase III gene; aac(3)-Ile</i>	Gentamicin	NODE.66_length_2854_cov.39.71171.1031/ISKpn19	100	100	GQ343134.1;CP125071;HCQ1792082.1
<i>aac(3)-IIa</i>	Gentamycin, tobramycin	NODE.66_length_2854_cov.39.71171.1031/ISKpn19	100	100	CP023555
<i>erm(B)</i>	Macrolide, lincosamide, streptogramin, quinupristin/dalfopristin	NODE.67_length_2837_cov.7.75646.420.1157	100;100	99.73;99.86	JN899585;CP082057
<i>aac(6)-Ib-cr</i>	Fluoroquinolone, ciprofloxacin, dibekacin, sisomicin, netilmicin, amikacin, tobramycin	NODE.70_length_2440_cov.46.4838.174.773	100	100	DO303918;GQ342986.1
<i>blaOXA-1</i>	Carbencillin, ampicillin, amoxicillin, piperacillin, ceftipime, ampicillin+clavulanic acid, amoxicillin+clavulanic acid, piperacillin+ tazobactam	NODE.70_length_2440_cov.46.4838.859.1734	100	100	HQJ07050;MN340011.1
<i>catB3</i>	Chloramphenicol	NODE.70_length_2440_cov.46.4838.1872.2420	70	100	U13889;AJ009818;KU544029.1