Genomic Ecology of ESBL-Producing *Escherichia coli* Across Human, Animal and Environmental Interfaces in Oman: A One Health Analysis of Resistance, Virulence and Plasmid Dynamics

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Article

Genomic Ecology of ESBL-Producing *Escherichia coli* Across Human, Animal and Environmental Interfaces in Oman: A One Health Analysis of Resistance, Virulence and Plasmid Dynamics

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Abstract

Background: Antimicrobial resistance is a One Health problem driven by the intricate interactions across human, animal, and environmental interfaces that enable microbial exchange and movement of mobile genetic elements encoding resistance and virulence. This study investigated the genomic epidemiology of ESBL and non-ESBL *Escherichia coli* across One Health interfaces in Oman. **Methods:** This prospective cross-sectional study analysed 245 non-duplicate *Escherichia coli* isolates from clinical, animal (diseased and healthy), sewage and water sources. Antimicrobial susceptibility testing was performed phenotypically, and a representative subset of ESBL and non-ESBL *Escherichia coli* from the three interfaces (n=50) underwent whole-genome sequencing to determine MLST, phylogroups, resistance genes, virulence determinants and plasmid replicons. **Results:** ESBL prevalence was highest in human isolates (73%), followed by sewage (28.6%) and animals (16.3% diseased; 8% healthy). *bla*CTX-M-15 predominated in humans, whereas *bla*CTX-M-55 dominated in animals and sewage, suggesting ecological partitioning with partial overlap. Quinolone resistance was least in the animal interface. Sewage isolates harboured the most complex resistome, including rmtB and plasmid-mediated quinolone resistance genes. MLST analysis revealed high diversity in human isolates, including globally recognised ExPEC lineages (ST10, ST38, ST73, ST127, ST131), while ST224 dominated in animals with evidence of possible spillover to humans. ST167 was confined to sewage, consistent with environmental maintenance of high-risk clones. Phylogroup structuring showed predominance of A, B2 and D among human isolates and A, B1, E among animal and sewage isolates. Virulence profiling demonstrated broader virulome diversity in humans, but shared core determinants (*fimH, sitA, traT*) across all domains. IncFIB(AP001918) was the dominant plasmid replicon, particularly among ESBL isolates, underscoring its role in horizontal gene dissemination. Alarmingly, mutation in pmrB (V161G) was identified in a healthy animal isolate pointing to a need for greater colistin restriction in animal husbandry. **Conclusions:** This study highlights plasmid-mediated resistance and shared virulence determinants linking reservoirs, although AMR profile was quite distinct across the three interfaces, with human isolates demonstrating greater resistance than animal isolates suggesting healthcare driven AMR in Oman. Continued integrated genomic surveillance is essential to monitor gene flow and inform coordinated antimicrobial stewardship strategies.

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1. Introduction

The growing recognition of the interconnectedness between human, animal, and environmental health has made the assessment of antimicrobial resistance (AMR) within the One Health framework exceedingly important. AMR is a quintessential One Health problem with the intricate integration of microbial exchange across the three interfaces allowing seamless movement of mobile genetic elements encoding resistance and virulence [1]. This global problem impacts not just human health but also animal health, food production, and environmental conditions [2].

Although many countries have drafted national action plans, implementation and dedicated budgets remain limited, underscoring the gap that One Health approaches aim to close. To narrow this gap the World Health Organization's (WHO) has introduced the Tricycle project which promotes multisectoral surveillance of Extended-Spectrum Beta-Lactamase (ESBL) carrying *Escherichia coli* [3].

E. coli is an ideal sentinel across the three interfaces: it is ubiquitous, easily cultured from clinical, veterinary, and environmental matrices, and frequently harbors plasmid-borne ESBL enzymes, like CTX-M/SHV/TEM. CTX-M alleles display setting-specific distributions—CTX-M-15 in human clinical isolates versus CTX-M-55 in bovine and wastewater sources—reflecting ecological selection and reservoirs. Increasingly, food-producing animals are being considered sources of ESBL *E. coli* in humans Ludden et al. have reported limited evidence linking AMR in human derived *E. coli* to livestock in their region [4–6].

Virulence genes are central to the disease-causing capacity of microbial pathogens, as they encode factors that enable organisms to adhere to hosts, establish colonization, invade tissues, evade immune defenses, and produce clinical manifestations of infection [7]. Increasing evidence indicates that virulence determinants frequently co-evolve and co-disseminate with antimicrobial resistance genes, particularly when they are carried on mobile genetic elements such as plasmids and transposons [8]. In the pandemic ST131 *E. coli*, the concurrence of virulence traits and antimicrobial resistance has been documented, indicating an alarming genetic convergence of virulence and resistance within this globally successful clone [9].

This study from Oman was a coordinated effort at sampling ESBL and non-ESBL *E. coli* from clinical care, diseased and healthy animals, sewage and water systems (falaj/wells), enabling side-by-side comparison of phenotype, genotype, and plasmid ecology. Whole genome sequencing (WGS) was carried out to assess and compare sequence types, resistance and virulence genes carriage, and plasmids aligning our study with One Health priorities: mapping gene flow among animals, humans, and the environment to quantify risks of transfer and to guide surveillance, stewardship, and WASH interventions.

2. Results

Antimicrobial Susceptibility Profile Across the Three Domains

The *E. coli* isolates from humans carried the highest burden of ESBL's, (73%) followed by sewage (28.6%) and diseased and healthy animals (16.3%, 8%). AmpC carriage was highest in human isolates (11.5%), followed by diseased animals (16.3%, 6.5%). CRE was restricted only to human isolates (4.8%). Isolates from water did not harbor ESBL, AmpC or CRE. (Supplementary Table S1) Detailed susceptibility profile of human and animal isolates is shown in Figure 1. As expected, susceptibility to third, fourth generation cephalosporins, and beta lactam-beta lactamase inhibitors were significantly lower in human isolates compared to animal isolates**;** the average third and fourth generation cephalosporin rates were 25% and 59% in the former while it was 71% and 87% respectively in the latter. Significantly, lower rates were observed in amoxicillin-clavulanic acid (39%)

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and piperacillin-tazobactam (61%) in human isolates while all healthy and diseased animal isolates were susceptible. In contrast, higher susceptibility to aminoglycosides were noted in human isolates (88% amikacin, 75% gentamicin) compared to animals (80% amikacin, 60% gentamicin) with streptomycin rates being lowest at 55% in animals. Surprisingly, enrofloxacin demonstrated superior rates than ciprofloxacin in animal isolates (90% and 93% in diseased and healthy isolates). Chloramphenicol demonstrated 100% susceptibility against human isolates compared to 75% against diseased animals respectively.

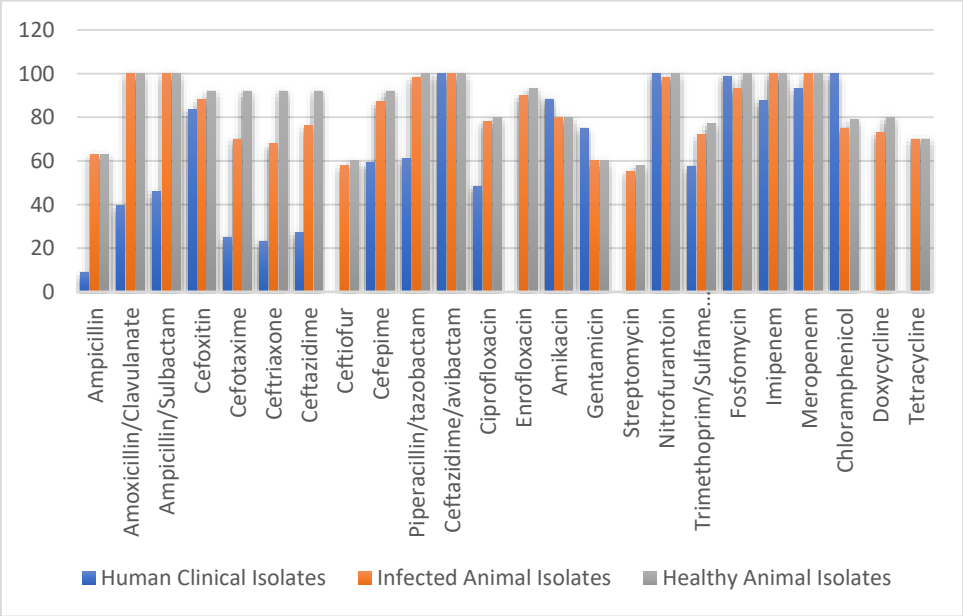

Figure 1. Antimicrobial susceptibility profile of *Escherichia coli* from human and animal clinical isolates and from healthy animals. The susceptibility rates of beta lactam antimicrobials were significantly lower in the human isolates compared to the animal isolates (Table 1); the average third and fourth generation cephalosporin rates were 25% and 59% in the former while it was 71% and 87% respectively in the latter. Similarly, lower rates were observed against beta-lactam-beta-lactam inhibitors in human isolates (39% for amoxicillin, clavulanic acid and 61% for piperacillin-tazobactam) while 100% rates were observed in both healthy and diseased animal isolates. Fluoroquinolones demonstrated similar results. Surprisingly, enrofloxacin demonstrated superior rates than to ciprofloxacin in animal isolates (90% and 93% in diseased and healthy isolates). In contrast, the reverse was true for aminoglycosides. Higher rates were noted in human isolates (88% amikacin, 75% gentamicin) and lower (80% amikacin, 60% gentamicin) in animals. Streptomycin susceptibility was even lower 55% and 58% in diseased and healthy animals. Surprisingly chloramphenicol demonstrated 100% susceptibility against human isolates compared to 75% and 79% against diseased and healthy animals respectively.

Distribution of AMR Genes and Mutations

Amongst the representative ESBL isolates sent for WGS, β-lactamase gene distribution differed across the domains. (Figure 2) In humans, *bla*CTX-M-15, (9/14) dominated, followed by 2/14 each of *bla*CTX-M-27 and *bla*CTX-M-55 while among 9/10 animal isolates 5 carried *bla*CTX-M-55 and 4 *bla*CTX-M-15 and all sewage isolates carried only *bla*CTX-M-55. *bla*DHA-1 (AmpC) was seen in only 3 human isolates co-harboured with *bla*CTX-M-15. Narrow-spectrum *bla*TEM-1B was co-carried in 6/14 human isolates and *bla*TEM-1A in 4/10 in sewage.

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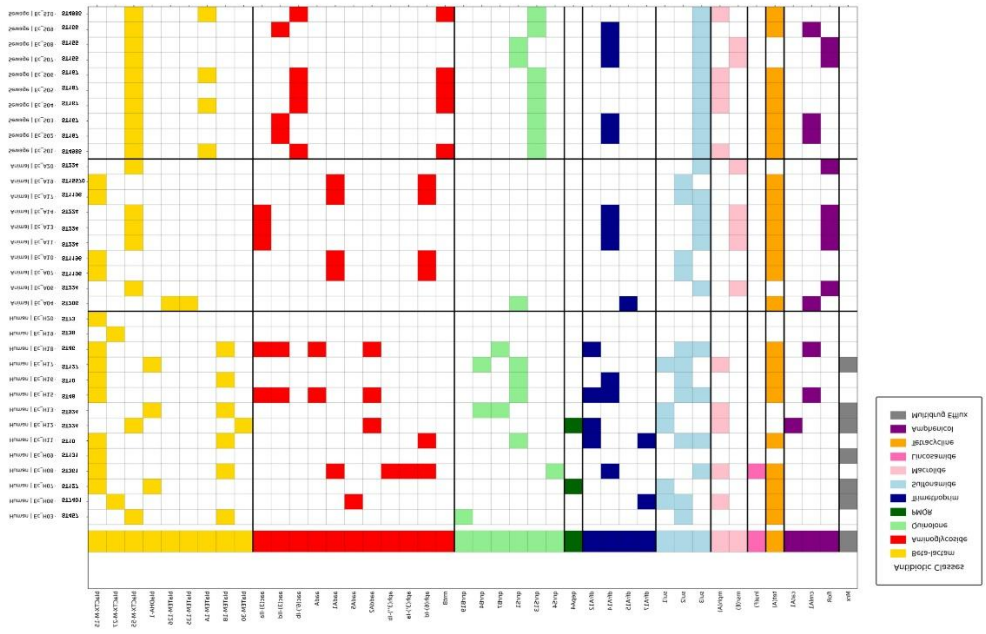

Figure 2. Heatmap Depicting Distribution of AMR Genes Across the Three One-Health Interfaces. In Humans the prevalence of *bla*CTX-M-15 was the highest, (9/14), followed by 2/14 each of *bla*CTX-M-27 and *bla*CTX-M-55. *bla*CTX-M-55 was found in 9/10 animal isolates, 5/10 animal isolates carried *bla*CTX-M-55 and 4/10 had *bla*CTX-M-15. In the sewage samples all the isolates carried *bla*CTX-M-55.

Diverse aminoglycoside resistance genes were detected across the interfaces: acetyltransferase genes *(aac(3)-IIa, aac(3)-IId), aac(6)-Ibl;* adenylyltransferase genes (*aadA1, aadA2,* aad*A5*), phosphotransferase genes (*aph(3')-Ib, aph(6)-Id*), and the 16S rRNA methyltransferase gene *rmtA*. Animal isolates demonstrated greater aminoglycoside resistance with 7/10 carrying one or two resistance genes; three carried *aac(3)-IIa* consistent with gentamicin, tobramycin and netilmicin resistance and four co-harboured *aadA* and *aph(6)-Id* consistent with streptomycin and kanamycin resistance). Sewage isolates displayed the most complex aminoglycoside resistome with 5/10 co-carrying *rmtB* which leads to pan aminoglycoside resistance and *aac(6)-Ib* (amikacin, tobramycin and netilmicin resistance). Fewer resistance was observed in humans. Two isolates co-harboured *aac(3)-IIa, aac(3)-IId, aadA1* and *aadA2* and one co-carried *aadA1, aph(3')-Ia, aph(3')-Ib, aph(3')-Id* and *aph(6)-Id.*

Quinolone resistance genes were observed in 8/14 humans, all sewage isolates and one animal isolate. Plasmid-mediated quinolone resistance (PMQR) genes predominated [*qnrS1* (4/14)] predominated in humans while 8/10 sewage isolates carried *qnrS13* and rest carried *qnrS1*.

Mutations conferring resistance to ciprofloxacin and nalidixic acid were detected in 28 isolates (8 from human clinical, 10 from animals and 10 from sewage), while mutations conferring resistance to colistin were found in only one isolate from animal (Table 1). All 28 isolates had *gyrA* mutations, *parC* in 26 and *parE* in 22 isolates. Among human isolates, six co-harbored *gyrA, parC* and *parE* mutations, while 2 had mutations only in *gyrA*. In animal isolates, nine co-harbored *gyrA, parC* and *parE* mutations, one co-harbored *gyrA* and *parC* mutations. Seven sewage isolates co-harbored *gyrA, parC* and *parE* mutations and 3 isolates *gyrA* and *parC* mutations. Interestingly a mutation in *pmrB* (V161G), was found in an healthy animal.

Table 1. Distribution of different mutations conferring antimicrobial resistance among the isolates from different domains.

Domain	Isolate	Gene	Mutation	Antibiotics
Human	Ec_H06	*gyrA*	S83L	Ciprofloxacin, Nalidixic acid
	Ec_H08	*gyrA*	S83L, D87N	

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Domain	Isolate	Gene	Mutation	Antibiotics
		parC	S80I	
		parE	S458A	
	Ec_H09	*gyrA*	S83L, D87N	
		parC	S80I, E84V	
		parE	I529L	
	Ec_H12	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_H15	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_H16	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	L416F	
	Ec_H18	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_H20	*gyrA*	S83L	
Animal	Ec_A04	*gyrA*	S83L, D87N	Ciprofloxacin, Nalidixic acid
		parC	S80I, E84G	
	Ec_A06	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A07	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A10	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A11	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A13	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A14	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A16	*pmrB*	V161G	Colistin
	Ec_A17	*gyrA*	S83L, D87N	Ciprofloxacin, Nalidixic acid
		parC	S80I	
		parE	S458A	
	Ec_A19	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_A20	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
Sewage	Ec_S01	*gyrA*	S83L, D87N	Ciprofloxacin, Nalidixic acid
		parC	S80I	

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Domain	Isolate	Gene	Mutation	Antibiotics
		parE	S458A	
	Ec_S02	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_S03	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_S04	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_S05	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_S06	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	
	Ec_S07	*gyrA*	S83L, D87N	
		parC	S80I	
	Ec_S08	*gyrA*	S83L, D87N	
		parC	S80I	
	Ec_S09	*gyrA*	S83L, D87N	
		parC	S80I, E84G	
	Ec_S10	*gyrA*	S83L, D87N	
		parC	S80I	
		parE	S458A	

A wider spectrum of trimethoprim resistance genes (*dfrA12, dfrA14, dfrA17*) were identified in human isolates, while *dfrA14* predominated in animal and sewage isolates. Sulfonamide resistance genes were among the most broadly distributed resistance determinants with *sul1, sul2, and sul3* being equally represented in humans, *sul2* and *sul3* in animals and *sul3* in sewage isolates. The macrolide resistance gene *mph(A)* was detected in human and sewage isolates, *mrx* gene exclusively in humans and *msr(E)* in animal isolates. Barring a few isolates tetracycline resistance gene *tet(A)* was present in all three domains. Amphenicol resistance genes (*cmlA1, floR*) were detected predominantly among animal and sewage isolates. One human isolate carried *catA1* and two *cmlA1.*

A narrow repertoire of antimicrobial resistance genes was detected among non-ESBL isolates compared to ESBL isolates. Although Resistance nodulation cell division (RND) efflux pump components were widely distributed across both ESBL and non-ESBL *E. coli*, ESBL isolates demonstrated a higher prevalence of efflux regulators and auxiliary efflux systems, including *marA, emrB, emrR, emrK* and *emrY* (Supplementary Table S2). For sulfonamide, trimethoprim, macrolides and quinolone resistance genes, only *sul1*, *dfrA17*, mrx and qnrB were detected, respectively, in non-ESBL clinical isolates and these were absent in non-ESBL animal isolates. However, non-ESBL animal isolates showed more diversity in RND genes and Major facilitator superfamily (MFS) genes compared to human non-ESBL isolates.

MLST Distribution

MLST distribution of *E. coli* was explored to assess convergence of transmission pathways across clinical, veterinary and environmental reservoirs (Figure 3, Table 2). No clear MLST pattern was observed across the three interfaces, with the human isolates demonstrating the greatest diversity (13 ST's overall; 11 in ESBL and 3 in non- ESBL with ST73 present in both). Clustering of four STs each were observed in animal and sewage ESBL isolates, with 9 additional STs observed in non-ESBL

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animal isolates. There was a clear separation of lineages between ESBL and non-ESBL *E. coli*, with occasional crossovers (ST73 and ST155).

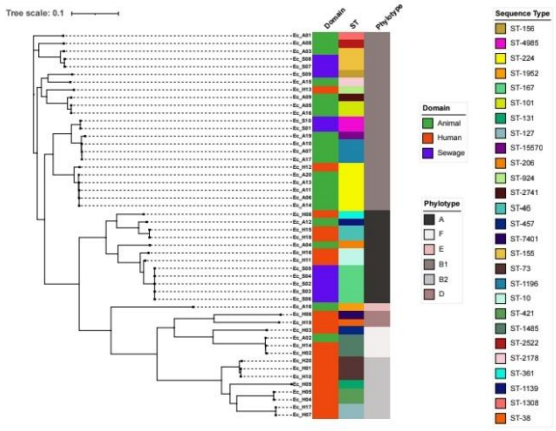

Figure 3. Phylotype structure and ecological distribution. No distinct MLST pattern was observed across the three interfaces (human, animal and environment). Highest diversity was observed in human isolates with 13 ST's altogether from 11 ESBL and 3 non-ESBL isolates. Four ST's were found to be clustered in isolates animal and sewage (ESBL producers). There was no distinction of lineages between ESBL and non-ESBL *E. coli*.

Table 2. MLST distribution of ESBL and non-ESBL *E. coli* across different domains of One Health.

MLST of Human derived *E. coli* Strains		**MLST of Animal derived *E. coli* Strains**		**MSLT of Sewage derived *E. coli* Strains**
Non-ESBL	ESBL	Non-ESBL	ESBL	ESBL
ST-73	ST-10	ST-101	ST-206	ST-155
ST-73	ST-10	ST-101	ST-224	ST-155
ST-421	ST-38	ST-155	ST-224	ST-156
ST-421	ST-46	ST-1139	ST-224	ST-167
ST-1485	ST-46	ST-1308	ST-224	ST-167
ST-1485	ST-73	ST-1485	ST-224	ST-167
	ST-127	ST-1952	ST-1196	ST-167
	ST-127	ST-2178	ST-1196	ST-167
	ST-131	ST-2522	ST-1196	ST-4985
	ST-224	ST-2741	ST-15570	ST-4985
	ST-361			
	ST-457			
	ST-924			
	ST-7401			

Overall, the ESBL population was characterized by 18 ST types. ST224 and ST1196 dominated in animals and ST167, ST155, ST4985 in the sewage while no striking pattern was noted in humans, although ST10, ST46 and ST127 featured in two each. Surprisingly, only one human isolate carried the global multidrug resistant clone ST131. A possible spillover of *bla*CTX-M-55 carrying ST224 was observed from animals to humans and non-ESBL ST155 from environment to animals (1 isolate each). The animal ST224 were characterized by the same resistance genes. In human isolates, ST38 and ST73 were remarkable for not carrying any resistance genes other than *bla*CTX-M27 and *bla*CTX-M15, respectively.

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The non-ESBL isolates were categorized into 11 STs of which only 3 STs (ST73, ST421 and 1485) were identified amongst human isolates. No distinct pattern was observed among animal non-ESBL *E. coli* isolates with only ST (ST101) occurring twice.

Phylotype structure and Ecological Distribution

Phylotyping revealed a clear pattern, with isolates spread over phylotypes A, B1, B2, D, E and F (Figure 3). Human isolates predominated in A, B2 and D. They formed distinct clades on the tree, representing specialized pathogenic lineages. In contrast, animal and sewage isolates were more frequently associated with phylotypes A, B1, and E, which are commonly linked to commensal strains and environmental persistence. Phylotype F isolates were less common but were observed within clusters containing both human and environmental isolates.

Serotyping

The in-silico analysis revealed distinct, partially overlapping serotype distributions although complete overlap at the O:H serotype level was limited (Table 3) Human isolates exhibited broader serotype diversity with O6 and O9 being the predominant variants, each comprising 25% (5/20) of the isolates. No significant H-antigen predominance was observed with H1 being most frequent (15%, 3/20), while H42, H7, H9, H18, H30, and H10 each represented 10% of isolates. A contrasting pattern was observed in animal isolates, with 30% (6/20) of O-antigens remaining untyped, with O78 dominating (25%, 5/20) followed by H23 (25%, 5/20), and H10 (20%, 4/20). Sewage samples demonstrated markedly lower diversity, with O101 and H10 representing 50% (5/10) each. Notably, O25:H4 serotype was detected in human isolate EcH09 (CTX-M-15 positive) and animal isolate EcA12 (O9:H4, non-ESBL producer), may be potentially related.

Table 3. Serotyping profile of isolates among the three domains based on O and H antigens.

Domain	Isolates	Serotyping		Domain	Isolates	Serotyping	
		H	O			H	O
Human	*Ec_H01*	H1	O6	Animal	*Ec_A01*	H31	O4
	Ec_H02	H42	O83		*Ec_A02*	H42	O83
	Ec_H03	H6	O11		*Ec_A03*	H25	O136
	Ec_H04	H7	O1		*Ec_A04*	H5	Unknown
	Ec_H05	H7	O1		*Ec_A05*	H40	O153
	Ec_H06	H18	O15		*Ec_A06*	H23	O78
	Ec_H07	Unknown	O6		*Ec_A07*	H10	Unknown
	Ec_H08	H30	O9, O9a		*Ec_A08*	H8	O185
	Ec_H09	H4	O25		*Ec_A09*	H12	O23
	Ec_H10	H1	O6		*Ec_A10*	H10	Unknown
	Ec_H11	H9	O9, O9a		*Ec_A11*	H23	O78
	Ec_H12	H30	O9a		*Ec_A12*	H4	O9
	Ec_H13	H21	O55		*Ec_A13*	H23	O78
	Ec_H14	H42	O83		*Ec_A14*	H23	O78
	Ec_H15	H10	O9a/O9		*Ec_A15*	H49	Unknown
	Ec_H16	H9	O101		*Ec_A16*	H28	Unknown
	Ec_H17	Unknown	O6		*Ec_A17*	H10	Unknown
	Ec_H18	H10	O9a/O9		*Ec_A18*	H40	O153
	Ec_H19	H18	O86		*Ec_A19*	H10	O109
	Ec_H20	H1	O6		*Ec_A20*	H23	O78
Sewage	*Ec_S01*	H23	O159	Sewage	*Ec_S06*	H10	O101
	Ec_S02	H10	O101		*Ec_S07*	H51	Unknown
	Ec_S03	H10	O101		*Ec_S08*	H51	Unknown

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	Ec_S04	H10	O101	*Ec_S09*	H28	O54
	Ec_S05	H10	O101	*Ec_S10*	H23	O159

Virulome Repertoire

Virulome profiling of ESBL producing *E. coli* revealed marked differences in distribution across the three interfaces (Table 4) although several virulence factors like curli production (*csgA*), type 1 fimbrial adhesion (*fimH*), serum resistance (*traT*), survival *(terC)* outer membrane protease (*ompT*), and key iron acquisition systems (*iroN, iucC, iutA, sitA*) were shared across human, animal, and sewage isolates.

Table 4. Distribution of virulence genes in ESBL producing *Escherichia coli* across the One Health Domains.

Virulence gene	Function	Human n/14, %	Animal n/10, %	Sewage n/10, %	Total n/34, %	NCBI Description
Adhesion/colonization genes,						
afaA	Adhesion/ Colonization	1/14, 7.14%	-	-	1/34, 2.94%	AfaVIII adhesin; member of afa-8 gene cluster; putative transcriptional regulator of the afa-8 gene cluster papI-papB family
air	Adhesion/ Colonization	1/14, 7.14%	-	-	1/34, 2.94%	
csgA	Adhesion/ Colonization	2/14, 14.29%	1/10, 10%	4/10 40%	7/34, 20.60%	Major subunit of curlin; it is actively secreted to the extracellular milieu, where CsgA monomers self-assemble into curli
fimH	Adhesion/ Colonization	8/14 57.14%	10/10 100%	10/10 100%	28/34, 82.35%	type 1 fimbriae D-mannose specific adhesin
focG	Adhesion/ Colonization	1/14, 7.14%	-	-	1/34, 2.94%	F1C minor fimbrial subunit protein G presursor
focIS	Adhesion/ Colonization	2/14, 14.29%	-	-	2/34, 5.88%	
iha	Adhesion/ Colonization	1/14, 7.14%	-	-	1/34, 2.94%	Adhesin Iha adhesin
ipfA	Adhesion/ Colonization	-	5/10, 50%	5/10, 50%	10/34, 29.41%	major fimbrial subunit IpfA,/Encodes a component of Long Polar Fimbriae in diarrheagenic and extraintestinal pathogenic E. coli ExPEC, straini
papC	Adhesion/ Colonization	4/14, 28.57%	-	1/10, 10%	5/34, 14.71%	Outer membrane usher P fimbriae
sfaD	Adhesion/ Colonization	3/14, 21.43%	-	-	3/34, 8.82%	S fimbrial/F1C minor subunit
sfaE	Adhesion/ Colonization	2/14, 14.29%	-	-	2/34, 5.88%	S fimbrial/F1C minor subunit

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sfaS	Adhesion/ Colonization	2/14, 14.29%	-	-	2/34, 5.88%	Sialic acid-binding adhesion
tia	Adhesion/ Colonization	-	-	1/10, 10%	1/34, 2.94%	Tia invasion determinant
yehC	Adhesion/ Colonization	-	-	2/10, 20%	2/34, 5.88%	putative fimbrial chaperone
yehD	Adhesion/ Colonization	-	-	2/10, 20%	2/34, 5.88%	fimbrial-like adhesin protein
Total adhesion/ colonization genes		**11(27)**	**3(16)**	**7(25)**	**68**	
			Bacteriocin genes			
cea	Bacteriocins	-	5/10, 50%	1/10, 10%	6/34, 17.65%	pore-forming bacteriocin colicin E1/Encodes for Colicin E7 and Dr adhesins bind to CEA
cia	Bacteriocins	-	-	9/10, 90%	9/34, 26.47%	colicin Ia protein
cib	Bacteriocins	-	1/10, 10%	4/10, 40%	5/34, 14.71%	Colicin ib/bacteriocin
cma	Bacteriocins	1/14, 7.14%	5/10, 50%	6/10, 60%	12/34, 35.29%	colicin M activity protein
colE8	Bacteriocins	-	3/10, 30%	-	3/34, 8.82%	Colicin E8 DNase,
cvaC	Bacteriocins	-	5/10, 50%	8/10, 80%	13/34, 38.24%	Microcin-V bacteriocin
mchB	Bacteriocins	1/14, 7.14%	-	-	1/34, 2.94%	Microcin H47/Bactericidal antibiotic.
mchC	Bacteriocins	1/14, 7.14%	-	-	1/34, 2.94%	MchC protein
mchF	Bacteriocins	1/14, 7.14%	-	-	1/34, 2.94%	ABC Type transporter activity/ATP binding and hydrolysis/Bacteriocin transport
Total bacteriocin genes		**4 (4)**	**5 (19)**	**5 (28)**	**51**	
			Immune evasion/survival genes			
Gad A	Immune Evasion/Survival	10/14, 71.43%	-	-	10/34, 29.41%	
Iss	Immune Evasion/Survival	6/14, 42.85%	-	-	6/34, 17.65%	increased serum survival lipoprotein Iss/ Resists the host's complement system, sepsis
KpsE	Immune Evasion/Survival	5/14, 35.71%	-	-	5/34, 14.71%	Capsule polysaccharide export inner membrane protein/Involved in the translocation of the polysialic acid capsule.

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kpsMII	Immune Evasion/Survival	5/14, 35.71%	-	-	5/34, 14.71%	capsular polysaccharide synthesis K1
ompT	Immune Evasion/Survival	6/14, 42.85%	9/10, 90%	1/10, 10%	16/34, 47.06%	Outer membrane protease protein protease 7,/Degrades antimicrobial peptides
Pic	Immune Evasion/Survival	1/14, 7.14%	-	-	1/34, 2.94%	Serine protease pic autotransporter
traT	Immune Evasion/Survival	5/14, 35.71%	10/10, 100%	8/10, 80%	23/34, 67.65%	complement resistance protein precursor TraT/Resists killing by the host's immune system serum resistance, by interfering with complement deposition and reducing phagocytosis
terC	Immune Evasion/Survival	3/14, %21.43	4/10, 40%	7/10, 70%	14/34, 41.18%	tellurium resistance membrane protein TerC
Total immune evasion/survival genes		**8/8 (42)**	**3/8 (23)**	**3/8 (16)**	**66**	
Iron acquisition genes						
chuA	Iron Acquisition	7/14, 50%	-	-	7/34, 20.59%	TonB dependent heme/hemoglobin receptor
fyuA	Iron Acquisition	8/14, 57.14%	-	1/10, 10%	9/34, 26.47%	ferric yersiniabactin uptake receptor FyuA
IreA	Iron Acquisition	1/14, 7.14%	-	-	1/34, 2.94%	TonB-dependent siderophore receptor IreA
iroN	Iron Acquisition	2/14, 14.29%	4/10, 40%	3/10, 30%	9/34, 26.47%	Enterobactin catecholate siderophore receptor protein/Encodes receptor which scavenges iron in iron poor environments
irp2	Iron Acquisition	3/14, 21.43%	-	-	-	High-molecular-weight protein 2 nonribosomal peptide synthetase
iucC	Iron Acquisition	2/14, 14.29%	10/10, 100%	2/10, 20%	14/34, 41.17%	Aerobactin synthetase
iutA	Iron Acquisition	1/14, 7.14%	6/10, 60%	3/10, 30%	10/34, 29.41%	Ferric aerobactin receptor
sitA	Iron Acquisition	10/14, 71.43%	10/10, 100%	8/10, 80%	28/34, 82.35%	iron/manganese ABC transporter substrate-binding protein/Transports ferrous iron and manganese
Total Iron acquisition genes		**8 (35)**	**4 (30)**	**4 (17)**	**82**	
aaiC	Secretion/Regulation	1/14, 7.14%	-	-	-	type VI secretion system protein AaiC/Hcp2
capU	Secretion/Regulation	2/14, 14.29%	-	-	-	putative hexosyltransferase CapU

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eilA	Secretion/Regulation	1/14, 7.14%	-	-	-	hilA-homologue/putative transcriptional regulator of ETT2 associated genes
etsC	Secretion/Regulation	-	-	1/10, 10%	-	Putative type I secretion outer membrane protein
hha	Secretion/Regulation	1/14, 7.14%	-	-	-	hemolysin expression-modulating protein Hha
traJ	Secretion/Regulation	2/14, 14.29%	-	2/10, 20%	4/34, 11.70%	Transfer of plasmid RP4 during bacterial conjugation requires the plasmid-encoded TraJ protein/Relaxosome protein
Total secretion/regulation genes		**5 (7)**	**-**	**2 (3)**	**10**	
			toxins/genotoxin genes			
astA	Toxins/Genotoxins	-	5/10, 50%	7/10, 70%	-	pAA
cibB	Toxins/Genotoxins	2/14, 14.29%	-	-	2/34, 5.88%	fratricide two-peptide bacteriocin subunit
cnf1	Toxins/Genotoxins	1/14, 7.14%	-	-	1/34, 2.94%	cytotoxic necrotizing factor 1
hlyA	Toxins/Genotoxins	1/14, 7.14%	-	-	1/34, 2.94%	Hemolysin A
hlyE	Toxins/Genotoxins	1/14, 7.14%	-	4/10, 40%	5/34, 14.7%	Hemolysin E
hlyF	Toxins/Genotoxins	2/14, 14.29%	-	6/10, 60%	8/34, 23.53%	cytoplasmic enzyme that increases the formation of outer membrane vesicles allowing the release of haemolysin E
USP	Toxins/Genotoxins	-	-	-	-	Uropathogenic-specific protein
Total toxins/genotoxin genes		**5 (7)**	**1 (5)**	**3 (17)**	**29**	
Total genes (total) isolates		**41 (122)**	**16 (93)**	**24 (106)**		

Colour scale

	10-39% prevalence
	40-59% prevalence
	60-79% prevalence
	80-100% prevalence

Human-derived isolates demonstrated a wider diversity of virulence-associated genes (41 genes), with the following groups dominating: adhesion and colonization (*afaA*, *sfa*, *foc*, *fim*, *iha*, *pap*, and *tia*), immune evasion (*kpsE*, *kpsMII*, *iss*, *traT*, and *ompT*), iron acquisition (*chuA, fyuA, iroN, irp2, iucC, iutA, sitA*), and toxicity (*cnf1, hlyA, hlyF, hlyE,* and *usp*). Animal-derived isolates exhibiting fewer (16 genes), shared the same iron uptake systems (*iroN, iucC, iutA, sitA*), and serum resistance proteins (*traT, ompT*). Sewage isolates carried 24 virulence genes, some common with either human and

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animal sources like adhesins (*csgA, fimH, ipfA, papC)* while *tia, yehC, yehD* were seen exclusively in sewage isolates. Both human and sewage derived isolates carried *fyuA*, *hlyE* and *hlyF*. Strains from animal and sewage shared *astA, cib, cvaC.*

On analysing genes present in more than 50% of ESBL isolates across the three domains, *fimH* (57.1%), *gadA* (71.4%), *chuA* (50.0%), *fyuA* (57.1%), and *sitA* (71.4%) predominated in human isolates. Animal derived *E. coli* were characterized by universal presence of *fimH, traT, iucC,* and *sitA* and high prevalence of *ompT* (90%), *iutA* (60%), and bacteriocin genes (*cea, cma, cvaC*). All sewage isolates carried *fimH* and majority carried *sitA* (80%), *traT* (80%), *terC* (70%), *cia* (90%), *cvaC* (80%), *cma* (60%), and *astA* (70%). Overall across the three domains, only *fimH* and *sitA* (both 82.4%) and *traT* (67.6%), were carried by majority of the isolates.

Plasmid Distribution

Overall, a total of 21 different plasmid replicon types were detected across the three domains; humans (11), animals (13), sewage (11) in ESBL and non-ESBL isolates. Amongst these IncFIB(AP001918) was the most prevalent plasmid replicon types (32/50, 64%), while IncFII(pRSB107), IncB/O/K/Z, ColpVC, Col440I and Col(pHAD28) were the least detected, each being detected once (1/50, 2%) (Figure 4).

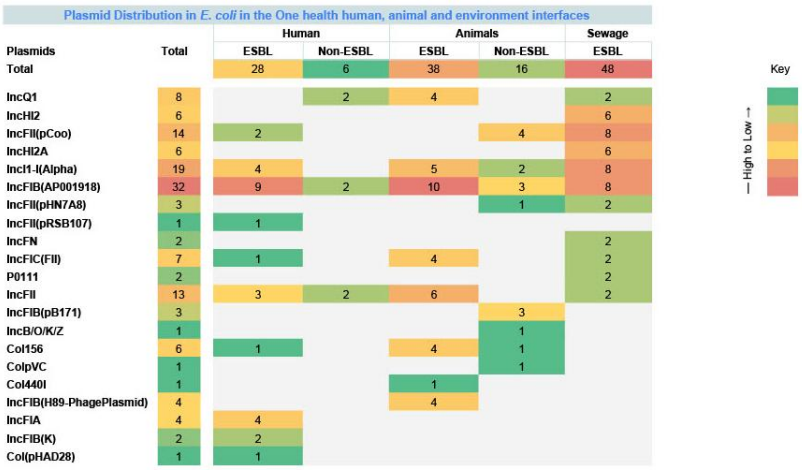

Figure 4. Comparative analysis of plasmid distribution in the three interfaces. Among the ESBL and non-ESBL isolates, 21 distinct plasmid replicon types were detected from Human (11), Animal (13) and Sewage Samples (11).

In human ESBL isolates (14), the most frequently detected plasmid replicon type was IncFIB(AP001918) (9/14, 64.29%), followed by Incl1-1(Alpha) and IncFIA (4/14, 28.57%), each. However, among non-ESBL isolates (6) only three plasmid replicon types were detected with identical frequency (2/6, 33.33%), each.

Similarly, in animal ESBL isolates, the most frequently detected plasmid replicon type was also IncFIB(AP001918) (10/10, 100%), followed by IncFII (6/10, 60%), Incl 1-1(Alpha) (5/10, 50%), then Col156, IncFIB(H89-PhagePlasmid), IncFIC(FII) and IncQ1 (4/10, 40%), each. Animal non-ESBL isolates (10) carried a wider portfolio of plasmids (8) compared to human non-ESBLs (3). The most detected plasmid replicon type among them IncFII(pCoo) (4/10, 40%), followed by IncFIB(AP001918) and (IncFIB(pB171) (3/10, 30%), each, then Incl 1-1(Alpha) (2/10, 20%).

Among all domains, the highest number of plasmid replicon types was detected in sewage isolates. The most frequently detected plasmid replicon types in sewage isolates were IncFIB(AP001918), Incl 1-1(Alpha) and IncFII(pCoo), which were detected among (8/10, 80%), each, followed by IncHI2 and IncHI2A (6/10, 60%), each, and the least plasmid replicon types were IncQ1, IncFII(pHN7A8), IncFN, IncFIC(FII), P0111 and IncFII (2/10, 20%), each.

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This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3. Discussion

A One Health approach provides valuable insights into the dynamic interconnections between human, animal, and environmental reservoirs that impact distribution of AMR, virulence traits, phylogenetic lineages, and mobile genetic elements. This study on One Health from Oman yielded many interesting insights.

The significantly higher burden of ESBL, AmpC and CPE in human *E. coli* in our study is noteworthy and consistent with global surveillance data. https://www.who.int/initiatives/glass. The absence of CPE in the other two interfaces suggests that carbapenem resistance in Oman is healthcare driven while aminoglycoside and chloramphenicol resistance reflects veterinary selection pressures. While *bla*CTX M-55 was common to all three interfaces, human isolates also carried *bla*CTX M-15, *bla*CTX M-27, and animals *bla*CTX-M15 while sewage only carried *bla*CTX-M-55, suggesting greater horizontal gene transmission between the latter two. Dominance of *bla*CTX-M15 in human isolates is consistent with its global presence in clinical ExPEC infections [10,11]. The carriage of only *bla*CTX-M55 in sewage *E. coli* and its dominance in animal isolates aligns with reports of increasing association with livestock, food chains, and environmental dissemination [12]. However, Yu et al. have reported a predominance of CTX-M55 in clinical isolates [13].

The presence of pan aminoglycoside resistance determinants, 16S rRNA methyltransferase *rmtB* and along with *aac(6')-Ib* in the sewage is extremely worrisome. Zhang et al. have reported similar findings in wastewater systems [14,15]. It is particularly concerning because they are frequently plasmid-borne and co-localise with ESBL genes, facilitating co-selection and persistence even when aminoglycoside exposure is intermittent [15].

A distinct PMQR gene distribution was observed with all sewage isolates carrying exclusively *qnrS13* (80%) or *qnrS1*(20%) and animals' stood out with least carriage while human isolates carried a varied set though *qnrS1* predominated. Banjo et al. reported higher prevalence of *qnrA* in hospital wastewater [16]. These findings support distinct pathways of dissemination of mobile quinolone resistance and point towards stepwise evolution toward high-level fluoroquinolone resistance [17,18]. The widespread presence of trimethoprim and sulfonamide resistance genes (*dfrA* variants and *sul1*/*sul2*/*sul3*) across domains reflects not only sustained antimicrobial exposure in both community and agricultural settings but also their association with mobile genetic elements [19]. Interestingly, there was blanket carriage of *tet(A)* across all domains while the amphenicol resistance genes (*floR*, *cmlA1*) were seen commonly in animal and sewage isolates. findings consistent with livestock-associated selection pressures and downstream environmental release. It is important to note that *floR* and *cmlA* are often found to be co-located with *tet* genes on the same plasmids [20].

Fluoroquinolone mutations (*gyrA*, *parC* and *parE)* though common, predominated in sewage isolates. Both human and animal isolates predominantly demonstrated mutations in all three genes. Point mutations were detected in the quinolone resistance determining region (QRDR) of the DNA gyrase (g*yrA*) as well as the DNA topoisomerase IV (*parC*) and (*parE*). However, no mutations were detected in (g*yrB*) DNA gyrase, a finding corroborated by previous studies [21,22].

This expanded efflux repertoire in ESBL strains compared to non-ESBL strains suggests enhanced possession of intrinsic resistance mechanisms that may act synergistically with β-lactamase production to promote multidrug resistance and persistence under antimicrobial pressure. The similar distribution of efflux-associated genes among human and animal non-ESBL *E. coli* isolates suggests conservation of these across reservoirs.

In this study, ST1952 isolated from a healthy animal from Muscat Governorate harbored *pmrB* mutation. To the best of our knowledge, this is the first report of *pmrB* mutation conferring colistin resistance from Oman in animals. Aworh et al. [22], reported a similar finding (V161G) from chicken in a poultry farm. Mutations in the *pmrAB* two-component regulatory system causes overexpression

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of certain bacterial operons (*pmrHFIJKLM* and *pmrCAB)*, which in turn results in colistin resistance by modification of the LPS structure [23].

Several important MLST lineages were identified (ST10, ST38, ST46, ST73, ST127, ST131, ST361, 155), many of which belong to well-described extraintestinal pathogenic *E. coli* (ExPEC) lineages associated with urinary tract and bloodstream infections in humans (e.g., ST131, ST73) or with food-animal and environmental reservoirs (e.g., ST10 complex, ST155) [24]. Co-existence of such globally recognized "high-risk" STs with more niche-specific STs (such as ST7401 or ST5713 in humans, ST1196 in animals and ST4985 in sewage) underscores the genomic plasticity of *E. coli* and the likelihood of both clonal expansion and horizontal gene transfer [25]. Overall, isolates within the same ST and interface, carried the same resistance genes.

Predominance of ST224 was observed amongst diseased animals, with a possible spillover to humans (one isolate), suggesting a genetic bridge between animal and human interfaces, all characterised by carriage of *bla*CTX-M55 which is often carried by an IncF plasmid [26]. ST224 is an international high-risk clone often associated with *bla*CTX-M variants, (*bla*CTX-M-15 and *bla*CTX-M-55), in poultry and human isolates [26]. This finding strengthens the possibility of food-producing animals, contributing ESBL-producing *E. coli* to humans either via direct contact, food chains or shared environmental sources [27]. However, it was noted in our study that the human ST224 had AMR genes distinct to that of animals' ST224. ST167 carrying *bla*CTX-M-55 and *rmtB*, dominated and were restricted to sewage isolates, suggesting that it may be primarily maintained in environmental or mixed human-waste reservoirs rather than in the sampled animal or clinical populations. However, Mujahid et al. has reported it in uropathogenic human isolates [28]. It belongs to the ST10 clonal complex, which is widely recognized as a host-generalist One-Health lineage found in humans, animals and environmental niches, frequently carrying *bla*CTX-M-15, other ESBLs and sometimes mcr-1 and carbapenemase genes [28]. The confinement of ST167 to sewage in our dataset is consistent with recent wastewater studies that demonstrate high diversity of ESBL-producing *E. coli* with over-representation of globally prevalent lineages such as the ST10 complex, ST38, ST69 and ST131 in wastewater and surface waters [29,30]. ST167 *E. coli* carrying *bla*CTX-M-55 and *bla*NDM-5 have been isolated from public environments, including municipal sewage, indicating potential waterborne transmission risks [30]. This supports the role of sewage as a mixing hub and amplifier of high-risk clones originating from multiple upstream sources.

The clear distinction between STs in non-ESBL and ESBL human and animal isolates suggests that they belong to distinct lineages. ST1485 and ST421 were largely confined to non-ESBL human isolates, and ST101 and several ST types in animal isolates, indicating circulation of specific low-resistance or commensal lineages in the community [31]. Notably, ST1485 has recently been recognized as a globally disseminated, high-risk, phylogroup F clone with zoonotic potential, frequently carrying ColV plasmids and multidrug resistance determinants [32,33]. Its presence here as non-ESBL type suggests its potential as a reservoir for acquiring ESBL and additional resistance genes over time, underlining the need for continued surveillance of apparently "susceptible" community lineages. Taken together, the ST patterns align with recent One-Health genomic studies which show only partial overlap between human and livestock ESBL-producing *E. coli* populations, with transmission often mediated by shared plasmids and mobile genetic elements rather than wholesale sharing of identical clones [6,34].

Phylotyping demonstrated clear ecological structuring, with human isolates clustering predominantly within phylogroups A, B2 and D, the latter two being classically associated with extraintestinal pathogenic *E. coli* (ExPEC) lineages and enhanced virulence potential [35]. Phylogroups A, B1 and E in animal and sewage isolates are commonly linked to commensal populations and environmental persistence, reflecting their broader ecological plasticity [36]. The presence of phylogroup F within mixed human–environmental clusters suggest potential cross-domain transmission, a pattern increasingly recognized in One Health genomic surveillance studies [6,24].

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Although human and animal domains exhibited a dominant serotype signature, the detection of O83:H42 in both domains suggests possible shared reservoirs. However, the limited number of identical serotype combinations indicates that direct cross-domain transmission may be less frequent than anticipated. Recent Australian One Health surveillance [37] has demonstrated that phylogenetic linkages at ≤100 SNP thresholds enable more accurate detection of cross-source transmission than serotyping alone. Although H10 was detected across all three domains, it was dominant in sewage. The ubiquitous presence of H10 across human, animal, and sewage domains aligns with Watt et al.'s (2025) demonstration of environmental compartments as key for *E. coli* lineage circulation in One Health surveillance [37]. The detection of O25:H4 (Ec_H09) is epidemiologically important. O25:H4 is strongly associated with the global multidrug-resistant ST131 lineage, a pandemic ExPEC clone responsible for urinary tract and bloodstream infections worldwide [38]. The ESBL-producing isolate of O25:H4/ST131 clonal group (Ec_H09) were CTX-M-15 producing. *E. coli* O25:H4/ST131 CTX-M-15 producing isolates have been reported in other countries [39].

The virulome patterns identified across human, animal, and sewage isolates underscore the multifaceted nature of ESBL-producing *E. coli* as a critical One Health pathogen [34]. The detection of core virulence determinants (*fimH, sitA, traT*) across all three interfaces supports the idea that *E. coli* maintains a conserved set of adhesins, iron uptake systems, and serum resistance genes reflecting their functional importance in diverse niches [40,41]. Abeni et al. too have reported the ubiquitous presence of *fimH* pointing to its evolutionary utility both within and across the interfaces [42]. Iron acquisition genes serve a valuable role in pathogenicity, with *sitA*, *iucC*, *iutA*, and *iroN* widely distributed across interfaces, supporting the growing evidence that these genes are central to the success of multidrug-resistant *E. coli* [43]. The presence of all 8 genes in the human isolates reflects the critical nature of iron scavenging systems in establishing ExPEC fitness ensuring survival in iron-limited as well as nutrient-poor environmental settings while the near universal presence of *sitA* underscores its role in establishing *E. coli's* versatility, findings corroborated by Gagaletsios et al. [44].

Human isolates possessed a wider virulome spectra (multiple adhesins, capsules, toxins, immune evasion/ survival genes and siderophore systems) compared to animal and environmental isolates pointing to greater selective pressures. One Health studies demonstrate that virulence gene diversity and abundance correlate with increased resistance burdens and clinical severity, further emphasizing the intertwined nature of virulence and multidrug resistance in extraintestinal *E. coli* [40]. Animal-derived isolates demonstrated high prevalence of serum resistance factors (*traT, ompT*) and aerobactin-mediated iron acquisition (*iucC, iutA*), enhancing survival in bloodstream and systemic infections. These are strongly associated with ExPEC plasmids raising concerns of livestock becoming potential reservoirs of virulence–resistance plasmids enhancing the potential of zoonotic transmission [24].

Sewage and animal isolates carried largely common bacteriocins (*cea, cib, cma, cvaC*) and immune evasion/ survival genes (*ompT, traT, terC*) with the bacteriocins being more abundant in the former while the reverse was largely true for the latter. The wastewater virulome reflects the intense microbial competition with bacteriocins providing a competitive advantage. The presence of this cocktail of genes alongside classical ExPEC determinants such as *fimH* and *traT* suggests the pivotal role wastewater systems may play in potentially reshuffling virulence and resistance traits via horizontal gene transfer [45,46].

In our study, IncFIB(AP001918), a conjugative plasmid replicon type was the most prevalent among the 21 plasmids across the domains in ESBL *E. coli*, being present in all animal isolates, 80% of sewage and in 64.29% of clinical isolates. Its dominant presence poses a grave threat as transmission of AMR and virulence genes across the three domains can be easily facilitated via horizontal transfer. IncFIB(AP001918) plasmids are highly stable, broad-host-range, and frequently carry ESBL genes (*bla*CTX-M-15), quinolone resistance (qnr), and virulence factors (e.g., siderophores), enabling one health transmission [47–49].IncFIB(AP001918) facilitates AMR spread from livestock/poultry to humans through food and environment [50].

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Interestingly, the non-ESBL animal isolates carried a larger repertoire of plasmids compared to human non-ESBL isolates. Not many studies have compared plasmids in non-ESBL animal and human isolates.

4. Materials and Methods

This prospective, cross-sectional collaborative One Health study, conducted from September 2023 to November 2024, characterized representative ESBL and non-ESBL *E. coli* across the three ecological interfaces (human, animal, and environment) in the Sultanate of Oman. The Department of Microbiology and Immunology, Sultan Qaboos University collaborated with Sultan Qaboos University Hospital, the Central Laboratory of Animal Health (CLAH), and Central Public Health Laboratory at Ministry of Health and NAMA Water Services. Ethical approval was obtained from the Medical Research Ethics Committee (MREC) at the College of Medicine & Health Sciences, SQU (REF.NO.SQU/EC/2678).

Sample Collection and Processing

From a total of 245 non-duplicate *E. coli* samples, 104 from 656 clinical isolates were from urinary tract, bloodstream, and respiratory tract infections, 123 from 259 isolates obtained from diseased animals (goats, sheep, cattle, camel, oryx and poultry), across different governorates in Sultanate of Oman (Supplementary Figure S1), 50 from 105 healthy animals, 14 from 35 sewage effluents and 4 from 40 samplings of falaj and wells (irrigation systems). All samples were collected, transported and processed according to standard protocols [51–54]. Detailed description of sewage and water sample collection and processing is provided in Supplementary File S1.

Bacterial identification was performed using MALDI-TOF MS (Bruker, Munich, Germany) for clinical isolates and at Central Analytical and Applied Research Unit (CAARU) at Sultan Qaboos University's College of Science for animal and environmental isolates. Only one isolate per patient or animal was included to avoid duplication. Antimicrobial susceptibility was performed by Phoenix™ (BD Diagnostics, Franklin Lakes, NJ, USA) at SQUH for clinical isolates and by Kirby Bauer disc diffusion method for animal isolates [55]. The antimicrobials tested by disc diffusion were ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin-tazobactam (10 µg), cefazolin, cefuroxime (30 µg), cefotaxime, ceftazidime (30 µg), ceftriaxone, cefepime (30 µg), and cefoxitin; carbapenems including imipenem (10 µg) and meropenem (10 µg); aminoglycosides including gentamicin (10 µg), amikacin (30 µg); fluoroquinolones including ciprofloxacin (5 µg) and levofloxacin; trimethoprim-sulfamethoxazole; tetracycline and doxycycline; and nitrofurantoin (30 µg).

ESBL was detected phenotypically in animal isolates by combined disk method, AmpC by disc approximation method in both human and animal isolates, and carbapenemase production by GeneXpert (Cepheid, Sunnyvale, CA, USA) in human isolates and lateral flow immunochromatographic assay (KPC/IMP/NDM/VIM/OXA-48 Combo Test Kit, Medomics, China) in animal isolates [56]. Sewage and water *E. coli* isolates were screened for ESBL and CRE carriage by ESBL and CRE CHROMagar (Paris, France). Confirmed isolates were stored at −20 °C in 50% glycerol until further analysis.

Whole Genome Sequencing and Bioinformatics Analysis

A representative subset of 50 ESBL-producing and non-ESBL *E. coli* isolates from human (14 ESBL and 6 non-ESBL), animal (10 ESBL from diseased and 10 non-ESBL from healthy animals), and sewage (10 ESBL) sources were selected for WGS. Sequencing was performed on the Illumina platform MicrobesNG, in the UK (https://microbesng.co.uk, Birmingham, UK) accessed on 12 September 2024. Isolates were processed using commercial extraction kits according to the manufacturer's protocol. DNA libraries were prepared following standard Illumina library preparation procedures and sequenced on an Illumina next-generation sequencing platform to generate paired-end reads. Raw sequence reads underwent quality control assessment, trimming,

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and de novo assembly. Assembled genomes were analyzed using various online tools from the Center for Genomic Epidemiology (CGE) (https://www.genomicepidemiology.org/) accessed on 18 November 2024 to identify Multi-Locus Sequence Types (MSLT) [57], acquired antimicrobial resistance genes (ResFinder and the Comprehensive Antibiotic Resistance Database (CARD), point mutations associated with antimicrobial resistance (ResFinder), plasmids replicons (PlasmidFinder), virulence factors (VirulenceFinder) and O:H serotypes (SerotypeFinder). A phylogenetic tree was constructed using CSI Phylogeny and the constructed tree was then visualized and annotated using Interactive Tree of Life (iTOL) [58] (accessed on 8 December2024). Additionally, [59], In Silico Clermont Phylotyper [59] (https://ezclermont.hutton.ac.uk/) was used for phylotype identification. All the genome sequences were submitted to NCBI, and accession numbers were awaited (submission ID: SUB16026812).

Statistical Analysis

Data was analyzed using IBM SPSS Statistics (Version 27). Categorical data were expressed as percentages. The sensitivity and specificity of phenotypic tests were assessed using chi-squared tests. Analysis of variance (ANOVA) was used to compare susceptibility profiles across different groups. Comparative analyses were conducted to evaluate differences in resistance prevalence across human, animal, and environmental sources. A p-value of less than 0.05 was considered statistically significant. All bar charts and pie charts were generated utilizing Microsoft Excel 2019. AI was employed to enhance a single graphic of the AMR heatmap.

5. Conclusions

The human interface was characterized by a clinically adapted, more complex resistome-virulome landscape, carrying the highest ESBL burden, a broader spectrum of AMR genes, and a richer virulome repertoire compared to animal and sewage isolates. Clinically relevant ExPEC-associated lineages and phylogroups predominated in the human domain, whereas animal and environmental isolates were more frequently associated with commensal-linked phylogroups and distinct resistance profiles. Although certain resistance determinants such as *bla*CTX-M-55 were shared across interfaces, the overall genomic landscape suggests limited direct cross-domain transfer of high-risk human-associated AMR and virulence genes within the sampled population. Across all domains, the virulome pattern displayed emphasizes the complex nature of ESBL-producing *E. coli* as a significant One Health pathogen. IncFIB(AP001918) was the predominant plasmid replicon type. A limited plasmid repertoire was observed in clinical non-ESBL isolates compared to animal non-ESBL isolates. Continued integrated genomic surveillance across One Health interfaces is essential to detect emerging convergence events and to inform coordinated stewardship, infection control, veterinary policy, and environmental management strategies.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial Resistance
ESBL	Extended-Spectrum Beta-Lactamase
non-ESBL	Non-Extended-Spectrum Beta-Lactamase
MLST	Multilocus Sequence Typing
ExPEC	Extraintestinal Pathogenic *Escherichia coli*
WHO	World Health Organization
WGS	Whole Genome Sequencing

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