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Original Article

Genomic profiling of extended-spectrum β-lactamase-producing *Escherichia coli* from Pets in the United Arab Emirates: Unveiling colistin resistance mediated by *mcr*-1.1 and its probable transmission from chicken meat – A One Health perspective

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ABSTRACT

Background: The United Arab Emirates (UAE) has witnessed rapid urbanization and a surge in pet ownership, sparking concerns about the possible transfer of antimicrobial resistance (AMR) from pets to humans and the environment. This study delves into the whole-genome sequencing analysis of ESBL-producing *E. coli* strains from healthy cats and dogs in the UAE, which exhibit multidrug resistance (MDR). Additionally, it provides a genomic exploration of the mobile colistin resistance gene *mcr*-1.1, marking the first instance of its detection in Middle Eastern pets.

Methods: We investigate 17 ESBL-producing *E. coli* strains from healthy UAE pets using WGS and bioinformatics analysis to identify genes encoding virulence factors, assign diverse typing schemes to the isolates, and scrutinize the presence of AMR genes. Furthermore, we characterized plasmid contigs housing the *mcr*-1.1 gene and conducted phylogenomic analysis to evaluate their relatedness to previously identified UAE isolates.

Results: Our study unveiled a variety of virulence factor-encoding genes within the isolates, with *fimH* emerging as the most prevalent. Regarding β-lactamase resistance genes, the *bla*CTX group 1 gene family predominated, with CTX-M-15 found in 52.9% (9/17) of the isolates, followed by CTX-M-55 in 29.4% (5/17). These isolates were categorized into multiple sequence types (STs), with the epidemic ST131 being the most frequent. The presence of the *mcr*-1.1 gene, linked to colistin resistance, was confirmed in two isolates. These isolates belonged to ST1011 and displayed distinct profiles of β-lactamase resistance genes. Phylogenomic analysis revealed close connections between the isolates and those from chicken meat in the UAE.

Conclusion: Our study underscores the presence of MDR ESBL-producing *E. coli* in UAE pets. The identification of *mcr*-1.1-carrying isolates warrants the urgency of comprehensive AMR surveillance and highlights the role of companion animals in AMR epidemiology. These findings underscore the significance of adopting a One Health approach to mitigate AMR transmission risks effectively.

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Introduction

Antimicrobial resistance (AMR) is a global health crisis that threatens the effectiveness of antibiotics, rendering once treatable infections increasingly difficult to manage [1]. One of the most concerning aspects of AMR is the emergence of multidrug-resistant bacteria at the human-animal interface, which has been pointed as a major One Health challenge [2]. The misuse and overuse of antibiotics in agriculture and veterinary practices can contribute to the emergence of resistant bacteria, which may then spread to humans through food consumption or close contact with livestock and companion pets [1,2]. Controlling the spread of antimicrobial resistance in these settings is crucial to safeguarding both animal and human health, highlighting the need for responsible antibiotic use and effective surveillance measures across the food and veterinary sectors [3].

Escherichia coli (*E. coli*), commonly residing in the digestive tracts of both humans and animals, possesses a multifaceted existence, ranging from a benign symbiotic microorganism to a potentially harmful pathogen. In its pathogenic state, it can lead to bacteremia, wound infections, urinary tract infections, and gastrointestinal tract infections [4]. Both non-pathogenic and pathogenic variants of *E. coli* have been associated with the dissemination of AMR genes to other bacteria, including those responsible for diseases in animals and humans [4]. Given its presence in a diverse array of hosts, *E. coli* serves as a valuable indicator for gauging the prevalence of antibiotic resistance, facilitating the evaluation and comparison of resistance rates across different populations and investigating the potential transmission from animals to humans [2].

In the realm of One Health, studies have consistently shown that close relationships and shared households between humans and their pet animals, such as cats and dogs, can significantly raise the risk of transmitting and spreading antibiotic-resistant bacteria between these humans and their animal companions [5,6]. Numerous investigations have identified the presence of Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli* strains in both healthy and ill dogs and cats. Furthermore, some studies have demonstrated the concurrent presence of ESBL-producing *E. coli* in dogs and humans living in the same households [6,7]. Additionally, resistance to colistin, mediated by mobile and plasmid-borne *mcr* genes, has emerged in pet animals across various countries, including the variants *mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4, *mcr*-5, *mcr*-8, *mcr*-9, and *mcr*-10 [8–10]. In the Middle East, there is limited research on this topic, with only one study from Egypt reporting the detection of *mcr*-9-positive *Enterobacter hormaechei* ST493 isolated from clinical samples obtained from dogs and cats [11].

The UAE is a country known for its rapid urbanization and economic growth, leading to changes in lifestyle and increased pet ownership [12]. While the interaction between humans and animals has many positive aspects, it also raises concerns about the potential transmission of antimicrobial resistance from pets to their owners and the environment. In a recent study from our group, high levels of ESBL-producing *E. coli* were found in healthy cats and dogs in the UAE, with the majority displaying multidrug resistance to critical antibiotics like fluoroquinolones and 3rd and 4th generation cephalosporins [12]. To tackle this concern, this study offers a thorough genomic analysis of ESBL-producing *E. coli* strains displaying multidrug resistance, which were obtained from healthy pets (both dogs and cats) in the UAE. Notably, it highlights the initial discovery of the *mcr*-1.1 gene in two separate *E. coli* isolates from unrelated healthy dog and cat. Recognizing the substantial One Health implications of this finding, these two isolates underwent a comprehensive bioinformatics examination, including an assessment of their phylogenetic ties to previously reported *mcr*-1.1-carrying isolates in the UAE context.

Materials and methods

Bacterial isolates

A collection of 17 previously confirmed ESBL-producing *E. coli* isolates, demonstrating resistance to three or more antimicrobial agents belonging to distinct classes (denoted as multidrug resistant (MDR)), were cultured from rectal swab samples collected from healthy cats and dogs admitted to veterinary clinics in Al Ain and Dubai cities of the UAE, during the period spanning from April to December 2022. Full details of the sampling approach and the initial epidemiological study is presented elsewhere [12]. These swabs were plated on Tryptone Bile X-glucuronide agar (Neogen, Lansing, USA) supplemented with ESBL agar additives (HiCrome ESBL Agar Supplement (FD278; HiMedia, Thane, India)). The ESBL agar supplement contained ceftazidime (1.5 mg), cefotaxime (1.5 mg), ceftriaxone (1.0 mg), aztreonam (1.0 mg), and fluconazole (5.0 mg) per vial for every 500 mL of medium, facilitating the selective growth of ESBL-producing *E. coli* strains. Isolates obtained from TBX-ESBL agar supplement plates were initially considered as potential ESBL producers, subsequently confirmed through E-test (Ezy MIC™ (HiMedia, Thane, India)), as per established protocols [12].

The confirmed ESBL-producing *E. coli* strains were further subjected to multiples PCR screening for β-lactamase genes, particularly *bla*CTX-M, *bla*TEM, *bla*SHV, and *bla*OXA [13]. Additionally, susceptibility testing for 12 antimicrobial agents was conducted using disc diffusion, and interpretation of results followed the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) [14]. The tested antibiotics, alphabetically ordered, included ampicillin (AMP (10 μg)), azithromycin (AZM (15 μg)), cefepime (FEP (30 μg)), cefotaxime (CTX (30 μg)), cefoxitin (FOX (30 μg)), ciprofloxacin (CIP (5 μg)), chloramphenicol (C (30 μg)), gentamicin (CN (10 μg)), imipenem (IPM (10 μg)), tetracycline (TE (30 μg)), trimethoprim-sulfamethoxazole (SXT (25 μg)), and ceftriaxone (CRO (30 μg)) [12]. Colistin resistance screening (defined as a minimum inhibitory concentration, MIC, value of ≥4 mg/L) was conducted using the broth microdilution method, as per established procedures [15].

Genomic characterization of ESBL-producing MDR E. coli from pets

Whole-Genome Sequencing (WGS) of 17 multidrug-resistant ESBL-producing *E. coli* strains was performed by the commercial sequencing facility, Novogene, using the Illumina NovaSeq platform PE150 (Illumina, San Diego, CA, USA). The bioinformatics analyses of the retrieved WGS data was processed as done before [4,15], in brief: the assembled sequences underwent species and serotype verification on PathogenWatch (accessed on April 17, 2023). In silico multilocus sequence typing was conducted following the *E. coli* Achtman scheme from PubMLST (accessed on April 17, 2023), and sequence types (STs) were determined using Enterobase (accessed on April 17, 2023). Acquired resistance genes were identified using ResFinder 4.1 with default parameters. Plasmid replicon types were determined with PlasmidFinder version 2.1, employing an identity percentage above 95% and a coverage cutoff exceeding 90%. The presence of virulence genes was assessed using VirulenceFinder version 2 with default parameters. SerotypeFinder 2.0 and CHTyper-1.0 were employed for serotype prediction and the detection of specific genes (*fumC* and *fimH*) for further categorization.

Genomic characterization of Colistin resistant E. coli from pets

Two unrelated *E. coli* isolates, each exhibiting a colistin minimum inhibitory concentration (MIC) of 4 mg/L and potentially reflecting a clinical resistance to colistin, besides being ESBL-producing and MDR, were identified. Recognizing the potential public health significance of such finding, in addition to being the first report for *mcr*-

1.1 isolation from pets in UAE and the Middle East, an exploration of their plasmid environments was undertaken. Plasmid contigs were extracted from the whole-genome sequences utilizing the PlasmidSPAdes tool (http://spades.bioinf.spbau.ru/plasmidSPAdes/) with default settings [16]. These reconstructed plasmid contigs were then aligned against the National Center for Biotechnology Information (NCBI) database to identify the most concorded plasmid matches. Furthermore, to investigate potential phylogenomic relationships, a phylogenomic analysis was carried out using CSI Phylogeny 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny) with default parameters, generating a maximum-likelihood phylogenetic tree [15]. This tree incorporated all *mcr*-1.1 harboring *E. coli* isolates from the UAE, including 47 from chickens, 3 from humans, and *E. coli* ATCC 25922 as a reference genome.

The whole-genome fastq files of the paired-end sequence reads for these isolates have been submitted via the National Center for Biotechnology Information (NCBI) and are publicly accessible under the BioProject accession number PRJNA938811.

Results

Virulence factors of MDR-ESBL-producing E. coli from pets

The whole-genome sequencing (WGS) of our study's isolates unveiled the presence of virulence factor-encoding genes, ranging from a minimum of 8 to a maximum of 38 (Table 1), which had been previously identified in *E. coli* strains isolated from healthy cats and dogs in the UAE. As presented in Table 1, among the characterized 17 *E. coli* isolates, the most commonly observed gene was *fimH*, found in 13 of the isolates, followed by *iss*, *sitA*, *terC*, and *traT*, each present in 10 isolates. Additionally, *nlpI* was detected in 9 isolates, while *iucC*, *iutA*, and *hlyE* were identified in 8 and 7 isolates, respectively (Table 1).

Genome-based typing of MDR-ESBL-producing E. coli from pets

Whole-genome sequencing and bioinformatic analysis revealed that the 17 *E. coli* isolates were assigned to 12 known sequence types (STs) (Table 2). The most frequently reported sequence type (3/17 (17.6%)) among the isolates was the pandemic *E. coli* sequence type 131 (ST131) (Table 2). This was followed by ST162 and ST1011, with two isolates each (Table 2). The WGS analysis inferred the presence of various serotypes among the study isolates. Notably, two isolates (ESBL22 and ESBL37) were identified as serotype O45, of which one isolate (ESBL37) was concurrently assigned as ST131 (Table 2). The majority of the isolates (7 out of 17) were associated with phylogroup B1, followed by phylogroup B2 (4 out of 17). All of the ST131 assigned isolates belonged to phylogroup B2 (Table 2). Regarding the genes responsible for type 1 fimbriae expression, both *fimH31* and *fimH41* were prevalent in our study collection, each appearing in three isolates per type (Table 2).

Genomic characterization of molecular markers associated with antimicrobial resistance

WGS analysis, as detailed in Table 3 and Fig. 1, revealed that the *blaCTX* group 1 gene family was the most frequently encountered, with CTX-M-15 present in 52.9% (9/17) of the isolates, followed by CTX-M-55 in 29.4% (5/17). Regarding the *blaTEM* gene family, the predominant allele was TEM-1B in 64.7% (11/17), which does not encode an ESBL-encoding β-lactamase gene (Table 3). Fig. 1 displays that six isolates exhibited macrolide resistance determinants, with *mph(A)* identified in four of them, and in combination with *erm(B)* and *mph(B)* in the other two isolates (Table 3). Concerning quinolone resistance, the *qnr* gene was observed in eight isolates, with seven carrying the *qnrS1* variant, and one (ESBL38) harboring *qnrB19*. Two isolates were found to possess the *ARR-2* gene, conferring resistance to rifampicin. The lincosamide resistance gene *lnu(F)* was detected in three isolates (Fig. 1). It is noteworthy that two isolates (ESBL32 and ESBL37) were found to contain the mobilized colistin resistance (*mcr*) gene *mcr*-1.1 (Table 3 and Fig. 1). Given the critical importance of colistin in human medicine, these two isolates underwent further detailed analysis.

Genomic features of mcr-1.1 harboring MDR-ESBL-producing E. coli from pets in the UAE

Both ESBL32 and ESBL37 isolates were classified as ST1011 within phylogroup D, but they exhibited distinct predicted serotypes. ESBL32 was associated with H16,O11 serogroup, while ESBL37 was predicted as H28,O45 (Table 3). Furthermore, these isolates displayed different profiles of β-lactamase resistance genes. ESBL32 carried blaCTX-M14b (an ESBL-β-lactamase) and blaCMY-2 (an AmpC-β-lactamase), whereas ESBL37 harbored blaCTX-M-8 and blaCTX-M-14b (ESBL-β-lactamase) (Table 3). Interestingly, the two

Table 1
Frequency of virulence genes in 17 multidrug resistant-ESBL-producing *E. coli* from pets in the United Arab Emirates.

Isolates code	Virulence-related genes	Frequency (n)
ESBL22	*AslA, chuA, fimH, fyuA, iha, irp2, iucC, iutA, kpsE, kpsMII_K5, nlpI, ompT, papA_F43, sat, senB, sitA, terC, traJ, traT, yehA, yfcV*	21
ESBL23	*cia, csgA, cvaC, etsC, fdeC, fimH, gad, hlyE, hlyF, iroN, iss, iucC, iutA, lpfA, mchF, nlpI, ompT, sitA, terC, traT, yehC*	20
ESBL24	*AslA, cba, cea, chuA, cma, colE8, eilA, fimH, gad, hlyE, hra, iha, iss, iucC, iutA, kpsE, kpsMII_K1, neuC, nlpI, ompT, papA_F19, sitA, terC, tia, yehD*	25
ESBL25	*AslA, chuA, fimH, fyuA, iha, irp2, iucC, iutA, kpsE, kpsMII_K5, nlpI, ompT, papA_F43, sat, senB, sitA, terC, traJ, traT, yehA, yfcV*	20
ESBL26	*cib, csgA, cvaC, etsC, fimH, gad, hlyE, hlyF, hra, iroN, iss, iucC, iutA, lpfA, mchF, nlpI, ompT, papC, sitA, terC, tia, traJ, traT, yehC*	24
ESBL27	*AslA, afaD, cea, chuA, clbB, cnf1, fimH, focC, fyuA, gad, hlyA, hra, iha, iroN, irp2, iss, iucC, iutA, kpsE, kpsMII, mchB, nlpI, ompT, papA_F43, papA_fsiA_F16, papC, sfaD, sitA, tcpC, terC, traT, usp, vat, yehA, yfcV*	38
ESBL28	*cea, csgA, fdeC, fimH, gad, hlyE, iss, lpfA, nlpI, sitA, terC, traJ, traT, yehD*	13
ESBL29	*cia, csgA, cvaC, etsC, fdeC, fimH, gad, hlyE, hlyF, iroN, iss, lpfA, mchF, nlpI, ompT, sitA, terC, traT, traJ, yehC, fyuA, irp2*	23
ESBL30	*cea, csgA, fdeC, fimH, gad, hlyE, iss, lpfA, nlpI, sitA, terC, traJ, traT, yehD*	14
ESBL31	*AslA, chuA, fimH, fyuA, iha, irp2, iucC, iutA, kpsE, kpsMII_K5, nlpI, ompT, papA_F43, sat, senB, sitA, terC, traJ, traT, yehA, yfcV, hha, hra*	24
ESBL32[a]	*chuA, cma, cvaC, eilA, etsC, fimH, hlyE, hlyF, hra, iroN, iss, iucC, iutA, nlpI, ompT, sitA, terC, traJ, traT, yehD*	19
ESBL33	*cib, csgA, cvaC, etsC, fimH, gad, hlyE, hlyF, hra, iroN, iss, iucC, iutA, lpfA, mchF, nlpI, ompT, papC, sitA, terC, tia, traJ, traT, yehC*	24
ESBL34	*AslA, astA, chuA, cia, csgA, cvaC, etsC, fimH, fyuA, hlyF, ireA, iroN, irp2, iss, iucC, iutA, lpfA, mchF, nlpI, ompT, pic, sitA, terC, traJ, traT, vat, yehA*	29
ESBL35	*eilA, fimH, gad, hlyE, iss, nlpI, ompT, terC, yehD*	8
ESBL36	*astA, cba, cma, csgA, cvaC, etsC, fimH, gad, hlyE, hlyF, iroN, iss, iucC, iutA, lpfA, nlpI, ompT, sitA, terC, traJ, traT, yehC*	23
ESBL37[a]	*chuA, cma, cia, cvaC, eilA, etsC, fdeC, fimH, hlyE, hlyF, iha, iroN, iss, iucC, iutA, nlpI, ompT, sitA, terC, traJ, traT, yehD*	24
ESBL38	*AslA, astA, cia, fimH, gad, hlyE, hlyF, iroN, iss, nlpI, ompT, sitA, terC, tia, traJ, traT, yehB*	17

[a] ESBL32 and ESBL37 coded isolates are those exhibiting colistin resistance (defined as a minimum inhibitory concentration, MIC, value of ≥4 mg/L).

Table 2
Whole-genome sequencing inferred typing characteristics of 17 multidrug resistant-ESBL-producing *E. coli* from pets in the United Arab Emirates.

Isolates code	Source	Multi locus sequence types	Serotype-finder Results	*FimH* allele type	CH Type: *4–31*	Phylogroup
ESBL22	Cat	131	H5, O45	*fimH41*	fumC40-fimH41:40–41	B2
ESBL23	Cat	345	H53, O134	*fimH31*	fumC4-fimH31: 4–31	B1
ESBL24	Cat	770	H51, O102	*fimH552*	fumC116-fimH552:116–552	E
ESBL25	Cat	131	H5, O25	*fimH41*	fumC40-fimH41:40–41	B2
ESBL26	Cat	162	H19, O9	*fimH32*	fumC65-fimH32:65–32	B1
ESBL27	Cat	12	H5, O4	*fimH386*	fumC13-fimH386:13–386	B2
ESBL28	Cat	8492	H9, O1	*fimH38*	fumC65-fimH38:65–38	B1
ESBL29	Cat	23	H9, O8	*fimH35*	fumC4-fimH35:4–35	C
ESBL30	Cat	8492	H9, O1	*fimH38*	fumC65-fimH38:65–38	B1
ESBL31	Cat	131	H5, O16	*fimH41*	fumC40-fimH41:40–41	B2
ESBL32	Cat	1011	H16, O11	*fimH31*	fumC4-fimH31: 4–31	D
ESBL33	Cat	162	H19, O9	*fimH32*	fumC65-fimH32:65–32	B1
ESBL34	Cat	117	H4, O33	*fimH97*	fumC45-fimH97:45–97	F
ESBL35	Cat	2614	H28, O160	*fimH1303*	fumC276-fimH1303:276–1303	B1
ESBL36	Cat	359	H21, O115	*fimH35*	fumC41-fimH35:41–35	B1
ESBL37	Dog	1011	H28, O45	*fimH31*	fumC4-fimH31: 4–31	D
ESBL38	Dog	2562	H45, O106	*fimH1080*	fumC54-fimH1080:54–1080	A

isolates shared three out of the four plasmid incompatibility types identified in each of them (Table 3).

Analysis of plasmid contigs extracted from WGS indicated that the *mcr*-1.1 gene was situated within a plasmid of approximately 61,589 bp for isolate ESBL32 and 62,013 bp for isolate ESBL37 (Fig. 2. A). This plasmid exhibited sequence similarity to a typical IncI2-type plasmid. A BLAST search of the NCBI database revealed other bacterial isolates with plasmids that were 99.9–100% identical to the plasmid sequences characterized in this study. Notably, *E. coli* plasmid pEGYMCR_IncI2 (with a coverage of > 94%), which was recovered from a chicken carcass in Egypt in 2018, and *E. coli* plasmid pMCR-GN775 (with a coverage of > 95%), obtained from polyclonal clinical *E. coli* strains in Argentina and Canada in 2019 (Fig. 2. B). Additionally, the virulence-associated gene *Hha*, which encodes a transcriptional regulator of known virulence genes, was located within the same contig (Fig. 2. B). Genetic mapping of the *mcr*-1.1 gene, as shown in Fig. 2. B, revealed the presence of the *PAP*-2 family protein-encoding gene downstream of *mcr*-1.1 in both ESBL32 and ESBL37 isolates. The *mcr*-1.1-*PAP*-2 element was flanked by a DNA topoisomerase gene and the *NikB* gene (Fig. 2. B).

To deduce the potential source of the two *E. coli* strains carrying the *mcr*-1.1 gene isolated from pets in this investigation, we conducted a phylogenomic analysis using available genomes of *E. coli* from the UAE known to harbor the *mcr*-1.1 gene (n = 47) and archived in the NCBI database. As illustrated in Fig. 3, both ESBL32 and ESBL37 were grouped within the same major branch, which also contained isolates from chicken meat in the UAE (Fig. 3). Examination of single nucleotide polymorphisms (SNPs) revealed that ESBL32 was closely related to those identified in a previous study in the UAE, specifically SRR20708246, SRR20708248, and SRR20708249, which were isolated from chicken meat and exhibited fewer than 265 SNPs. Additionally, ESBL37 displayed a close genetic relationship with SRR20708252, obtained from retail chicken in the UAE in 2021, differing by only 16 SNPs.

Discussion

In this study, we conducted a comprehensive genomic analysis of multidrug-resistant ESBL-producing *E. coli* strains isolated from healthy cats and dogs in the UAE. Our research represents the initial in-depth genomic investigation into antimicrobial resistance among companion animals in the UAE and the Gulf Cooperation Council nations. The study revealed intriguing results: (i) comprehensively identify genes responsible for virulence factors, (ii) provide genome-based typing of the isolates, (iii) elucidate the mechanisms behind antimicrobial resistance in these strains, and (iv) particularly focus on characterizing the genetic context and phylogenetic relationships of colistin-resistant isolates carrying the *mcr*-1.1 gene. This contributes valuable insights to the broader regional context of One Health, emphasizes the often underestimates significance of companion animals in the context of antimicrobial resistance epidemiology in the Middle East [7,10].

The analysis of virulence factors in the characterized *E. coli* strains from healthy pets in this study highlights the prevalence of genes associated with both extra-intestinal and intestinal pathotypes. The *fimH* gene encodes the FimH protein, an essential component of type 1 pili responsible for mediating bacterial adhesion to host tissues, facilitating colonization [17]. Meanwhile, the *iss* gene is linked to increased survival in the bloodstream, allowing *E. coli* to evade complement-mediated killing and potentially leading to systemic infections upon transmission [18]. Detection of the *terC* gene in pet animal fecal samples may indicate *E. coli* strains adapted to environments with elevated tellurium levels, reflecting their adaptability and potential for environmental persistence [19]. The *nlpI* gene encodes a non-specific nuclease involved in DNA degradation, which could contribute to tissue invasion and immune evasion [17]. Furthermore, the *hlyE* gene encodes a pore-forming hemolysin, enabling *E. coli* to produce a substance that can harm host cells and enhance virulence [20]. Therefore, the notable presence of these virulence factors in our study isolates underscores the imperative for implementing a One Health antibiotic stewardship program and establishing coordinated surveillance to monitor the spread of antimicrobial resistance across the human-animal interface in the UAE.

In this study, the genomic analysis of isolates from healthy pets in the UAE uncovered several characteristics that may present a potential threat to human health, particularly the identification of ST131 and the O45 serotype. ST131, the most common ST among the study isolates, has been linked to the global dissemination of ESBL-resistance genes. Added to that, ST131 plays a significant role in urinary tract infections, both in healthcare settings and the community, along with bloodstream infections and infections affecting companion animals and poultry [21,22]. It's important to note that the prevalence and significance of ST131 can vary by region and over time. This is the first report of ST131 *E. coli* in animals in the UAE, and finding such high-risk clone in healthy pets is worrisome given their close contact and household sharing to their owners. Additionally, we report in this study a genome-based evidence on the presence of the O45 serotype, one of the leading six non-O157:H7 Shiga toxin-producing *E. coli* (STEC) serotypes [23]. Such pathogenic serotype has been recognized as a causative agent of sporadic instances of bloody diarrhea in humans [23,24]. The fact that one of the three *E. coli*

Table 3
Whole-genome sequencing inferred characterization of plasmid incompatibility types and antimicrobial resistance genes among 17 multidrug resistant-ESBL-producing E. coli from pets in the United Arab Emirates.

Isolates code	Source	Plasmid Incompatibility type (Plasmid finder)	Polymyxin resistance	Beta-lactam resistance	Macrolide resistance	Tetracycline resistance	Sulphonamide resistance	Trimethoprim resistance	Phenicol resistance
ESBL22	Cat	IncFIB, IncFII	x	*blaCTX-M-15, blaTEM-1B*	x	x	x	x	x
ESBL23	Cat	IncFIB, IncFII, Incl1	x	*blaCTX-M-15, blaTEM-1B*	*mph(A)*	*tet(A)*	*sul2*	x	*floR*
ESBL24	Cat	p0111	x	*blaCTX-M-55, blaTEM-1 C*	x	*tet(A)*	*sul1, sul2*	*dfrA1*	*floR*
ESBL25	Cat	IncFIA/IncFIB, IncFII	x	*blaCTX-M-15, blaTEM-35*	*mph(A), erm(B)*	x	x	x	x
ESBL26	Cat	Incl1, IncFIB, IncFII	x	*blaCTX-M-15, blaTEM-1B*	x	*tet(A)*	*sul2*	*dfrA14*	x
ESBL27	Cat	IncFIA, IncFIB	x	*blaCTX-M-15, blaTEM-1B*	*mph(A)*	x	*sul2*	*dfrA14*	x
ESBL28	Cat	IncHI2, p0111, Incl1, Incl2	x	*blaCTX-M-55, blaTEM-1B, blaLAP-2*	x	*tet(A)*	*sul3*	*dfrA14*	*floR*
ESBL29	Cat	IncFIB, IncFII	x	*blaCTX-M-15, blaTEM-1B*	x	*tet(A)*	*sul2*	*dfrA5*	x
ESBL30	Cat	IncHI2, p0111, Incl1, Incl2	x	*blaCTX-M-55, blaTEM-1B, blaLAP-2*	x	*tet(A)*	*sul3*	*dfrA14*	*floR*
ESBL31	Cat	IncY, IncFII	x	*blaCTX-M-15, blaTEM-1B*	*mph(A)*	*tet(A)*	*sul1, sul2*	*dfrA17*	x
ESBL32	Cat	Incl2, IncFIB, IncHI2A, IncHI2	*mcr-1.1*	*blaCMY-2, blaCTX-M14b*	*mph(A), mph(B)*	*tet(A)*	*sul1, sul2, sul3*	*dfrA1*	*cmlA1, floR*
ESBL33	Cat	Incl1, IncFIB, IncFII	x	*blaCTX-M-15, blaTEM-1 C*	x	*tet(A)*	*sul2*	*dfrA14*	x
ESBL34	Cat	IncFIB, IncN, IncFII	x	*blaCTX-M-55*	x	x	x	x	x
ESBL35	Cat	x	x	*blaCTX-M-55*	x	*tet(A)*	*sul2*	*dfrA17*	*floR*
ESBL36	Cat	IncFIB, IncX1, IncFIB	x	*blaCTX-M-15, blaTEM-1B*	x	*tet(A)*	*sul1, sul2*	*dfrA1*	x
ESBL37	Dog	IncHI2, Incl1, Incl2, IncFIB	*mcr-1.1*	*blaCTX-M-8, blaCTX-M-14b, blaTEM-1B*	*mph(A)*	*tet(A)*	*sul1, sul3*	*dfrA12*	*catA1, cmlA1*
ESBL38	Dog	Incl1, IncX1, IncFIB	x	*blaCTX-M-2, blaTEM-1B*	x	*tet(A)*	*sul1, sul2, sul3*	*dfrA12*	*cmlA1, floR*

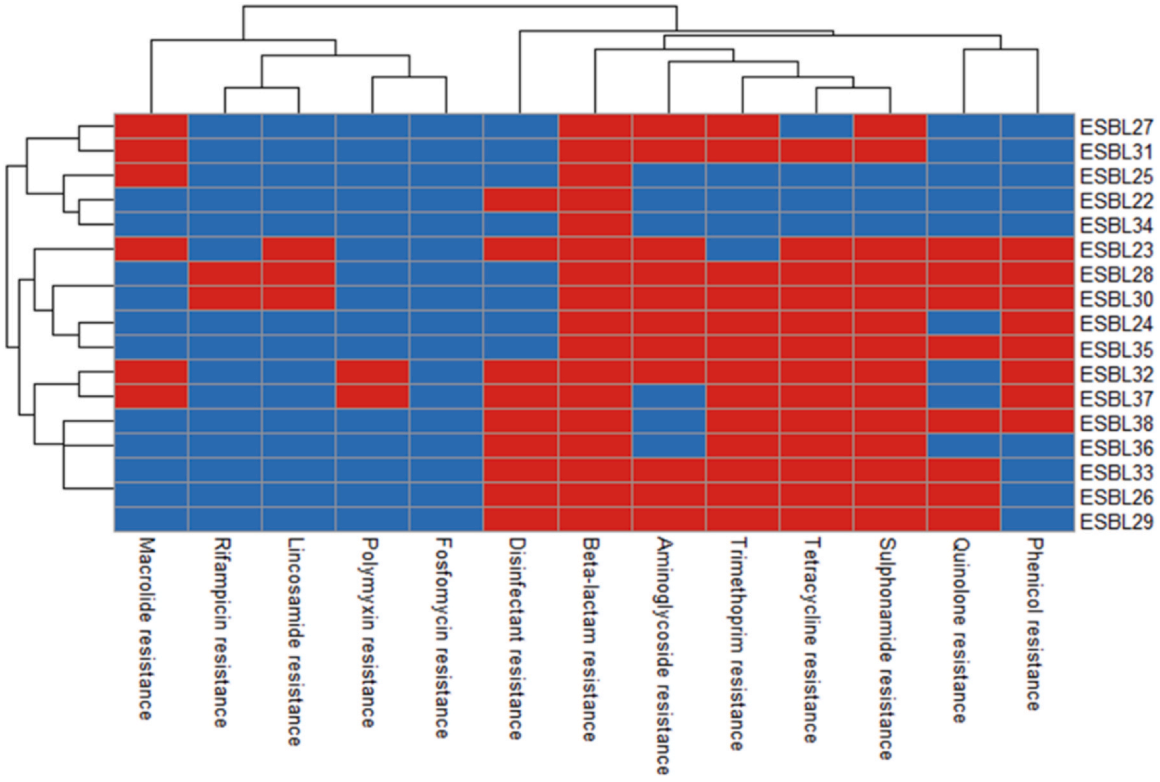

Fig. 1. Heatmap showing hierarchical clustering of antimicrobial resistance gene profiles in 17 multidrug resistant-ESBL-producing *E. coli* from pets in the United Arab Emirates. Red tiles indicate presence of antimicrobial resistance genes; blue tiles indicate absence of resistance gene.

isolates identified as ST131 was also serotype O45 implies that such isolate might carry various pathogenic potentials. This significance finding becomes more pronounced when considering the isolate potential as MDR. Monitoring and understanding the dynamic of high-risk *E. coli* clones at the human-animal interface are crucial for the development of effective infection control strategies [25].

The clinical significance of bacterial species does not solely rely on the mere presence of resistance genes and other mechanisms. Rather, the presence of these genes could facilitate the emergence of clinical relevance. Moreover, these genes possess the potential to be transferred from one species to another, thereby giving rise to new resistance patterns in commensal or pathogenic species that share a common host or environment [1,2]. In our research, we observed a high occurrence of the *blaCTX* group 1 gene family in ESBL-producing *E. coli* strains obtained from healthy pets in the United Arab Emirates (UAE). For the first time, we identified genes, particularly CTX-M-15, that are linked to ESBL-producing *E. coli* strains in healthy pets in the UAE. These *E. coli* strains producing CTX-M-15, which are commonly found in humans, are appearing as emerging β-lactamase-producing strains in veterinary species, including unwell dogs and cats [26,27]. The presence of *E. coli* strains that produce CTX-M-15 in healthy cats and dogs raises concerns for public health, as there is a potential risk of these antibiotic-resistant strains being transmitted between companion animals and humans [26,27]. Given this, and considering the significant detection in the present isolates collection of various groups of antimicrobial resistance genes, including plasmids carrying such genes, we recommend that pet owners take measures such as properly disposing of dog feces and practicing hand hygiene, which can include using a hand sanitizer, to reduce the risk of transmission.

This study marks a significant milestone for the Middle East, as it presents the first-ever identification and characterization of two *E. coli* strains carrying IncI2-type plasmids with the *mcr-1.1* gene and displaying ESBL-producing capabilities. In the Middle East region, research on this subject is scarce, and only one study conducted in Egypt has documented the identification of *mcr*-9-positive *Enterobacter hormaechei* ST493 isolated from clinical samples obtained from dogs and cats [11]. Intriguingly, both of these *mcr*-1.1-carrying isolates identified in our study, despite originating from different cities in the UAE and being obtained from a dog and a cat, shared the same ST types, ST1011. Notably, in a study conducted in China by Jiang et al.[28] it was also observed that among four *E. coli* carrying the *mcr*-1 gene, two of them, sourced from dogs and cats in Shanghai and Beijing, belonged to the identical ST (ST1011).

In previous research within the UAE, ST1011 had been identified as one of the most prevalent STs among ESBL-producing multidrug-resistant *E. coli* strains recovered from chilled whole chicken carcasses sampled in supermarkets [4]. Furthermore, our phylogenomic analysis revealed that the two *mcr*-1.1. carrying *E. coli* isolates from pets have a close genetic relationship with isolates from chicken meat in the UAE. The presence of only 16 single nucleotide polymorphism (SNP) differences between one of our study's isolates (ESBL37, from a dog) and a chicken meat isolate suggests a potential transfer of *mcr*-harboring *E. coli* between pets and chicken meat isolates. Hypothetically, this transfer might have been occurred through human (owner) to pet transmission, or through environmental exposure. With regard to environmental exposure, we noted in a previous study in the UAE that cats and dogs with access to water in ditches and puddles have higher likelihood to be positive to ESBL-positive *E. coli* than those without access to open water sources [12]. Nevertheless, the genomic tools we used in this study emphasis that transmission scenario through the consumption of raw chicken by pets or via pets coming into contact with an environment contaminated with *E. coli* carrying the *mcr*-1.1 gene originating from chicken. Our investigation also unveiled that the plasmids carrying the *mcr*-1.1 gene in one of the *E. coli* isolates obtained from healthy dogs and cats in the UAE shared a nucleotide sequence identity of ≥ 99.5% with a plasmid found in *E. coli* sourced from chickens in

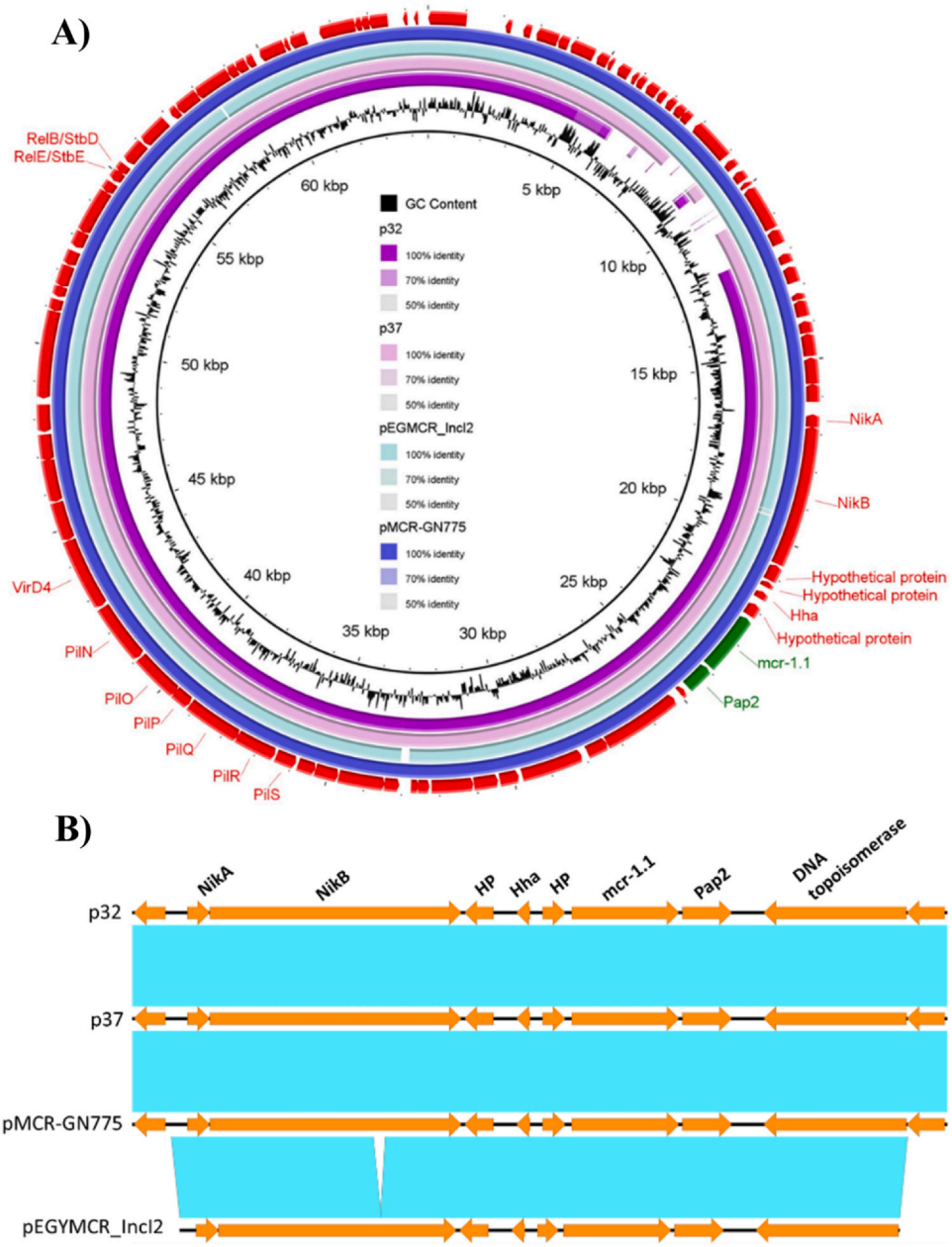

Fig. 2. Circular alignments of reference plasmid sequences with homologous *mcr*-1.1 carrying contigs from pet originated *E. coli* characterized in this study. (a) comparison of reconstructed *mcr*-1.1 positive plasmid of isolates ESBL32 and ESBL 37 with plasmids pMCR-GN775 and pEGMCR_Incl2. (b) Linear comparison of the *mcr*-1.1 harboring region showing the gene context comparison between *E. coli* isolates ESBL32 and ESBL 37 with pMCR-GN775 and pEGMCR_Incl2. Blue shading in the linear maps denotes regions of shared homology among different plasmids with 100% similarity.

Egypt. This finding, combined with the fact that previous studies have identified the *mcr* gene in pet foods primarily composed of chicken [29,30], strengthen the potential hypothesis that the detection of the *mcr*-1.1 gene in *E. coli* from companion animals might have originated from chicken meat included in their diet as pet food.

The prevalent plasmid types known for carrying the *mcr*-1 gene, such as IncI2, IncX4, Inc, and IncHI2, have been documented in previous publications [31]. In our study, the *mcr*-1.1 genes were found on IncI2 plasmids, which have been identified in *E. coli* isolates sourced from food, livestock, and humans in several countries [32–34]. The plasmid reconstruction analysis of the genetic context surrounding the two *E. coli* strains carrying the *mcr*-1.1 gene revealed that this gene was incorporated within a gene arrangement containing *PAP2*/*mcr*-1.1/DNA topoisomerase. This configuration mirrors findings in other comparative genomic analyses of polymyxin-resistant *Enterobacteriaceae*, where a gene encoding a *PAP2* superfamily protein consistently resides downstream of *mcr*-1 [15]. This observation reaffirms that *PAP2* play a crucial role in the functioning of *mcr*-1. Furthermore, it's worth noting that the plasmid carrying the *mcr*-1.1 genes in our current study also included a copy of the transcriptional regulator *hha* gene. This regulator gene has previously been identified on both the chromosome and plasmid of *E. coli O157:H7* [35]. While initially recognized as a regulator of hemolysin production, the *hha* gene has since been associated with the regulation of pathogenicity and the formation of biofilms [36]. These findings emphasize the importance of conducting further sequence-based characterization of antibiotic resistance plasmids found in *E. coli* isolated at the interface between humans and animals.

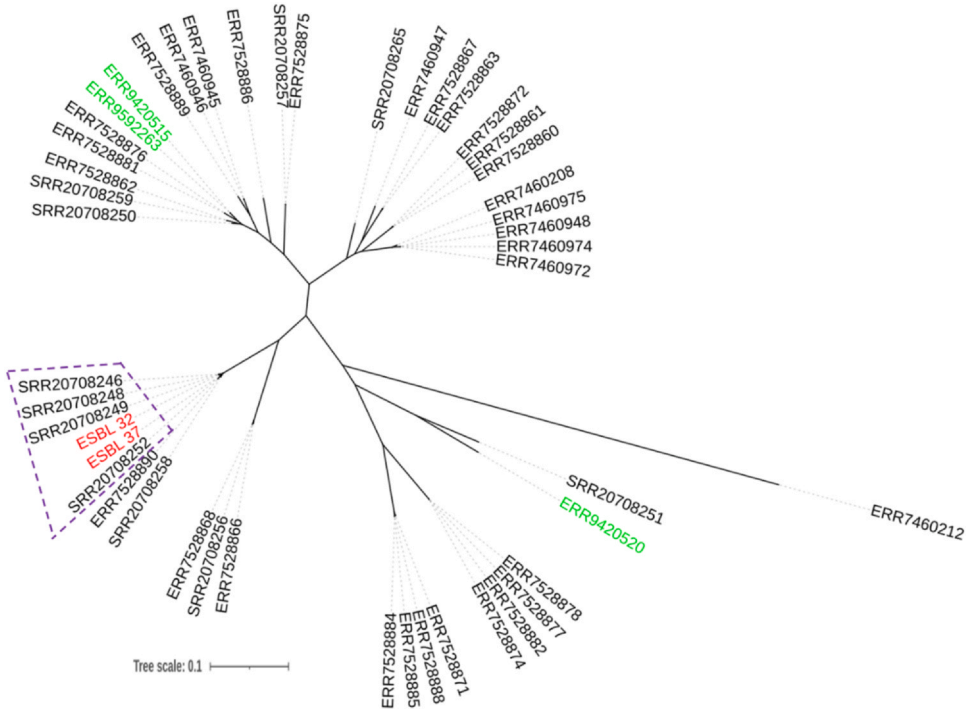

Fig. 3. Phylogenetic tree highlighting the position of *mcr*-1.1 harboring ESBL-producing *E. coli* (ESBL32 and ESBL 37, in red) isolated from pets relative to other *mcr*-1.1 harboring *E. coli* Isolated from in the United Arab Emirates (n = 47 (44 from chicken (in black) and 3 from Humans (in green)). The whole genome SNP based phylogeny was established with CSI phylogeny version 1.4 using *E. coli* ATCC 25922 as a reference and standard input parameters.

Conclusion

Our findings notably confirm the presence of *mcr*-1.1-carrying *E. coli* among companion pets in the UAE. However, it's crucial to note that due to the preliminary nature of the initial study from which our isolate collection was derived, our results cannot accurately represent the overall prevalence of *mcr* genes in companion pets across the UAE. Given that *E. coli* has zoonotic potential [37], a thorough epidemiological and microbiological inquiry into these strains is necessary to pinpoint reservoirs and potential transmission routes. Our genomic evidence suggests a significant connection of the isolates from pets to chicken sources, possibly through direct exposure via meals or environmental contact. Therefore, further comprehensive investigations into the transmission of *mcr*-1.1 in UAE pets, considering the 'One Health' approach, are imperative. National policy for combating AMR in the UAE will benefit from improving infection control practices and antimicrobial stewardship at the broader veterinary sector.

Ethics approval and consent to participate

The study was approved by the United Arab Emirates University Animal Research Ethics Committee (Permit number: ERA_2022_8520).

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CRediT authorship contribution statement

Conceptualization, I.H.; data curation, M.E., and A.G.; formal analysis, I.H., M.-Y.I.M., A.A., G.B.L., F.A.; investigation, K.M. and F.A.; methodology, I.H., M.E., A.G., H.K.; project administration, H.K, K.M. and M.K.; resources, M.E., and A.G.; supervision, I.H.; writing— original draft, I.H, M.B, A.G.; writing—review and editing, H.K., M.K., K.M., M.-Y.I.M., F.A., G.B.L., A.A. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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