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Whole-Genome Analysis of an Extensively Drug Resistant Enterobacter Hormaechei Clinical Isolate from Mosul, Iraq

Sahar Salim Petrus Al-Nakkar[1], Amerah Ali Ahmed[2], Enas Abdul Munieem Al-Layla[3] and Essra Ghanim Hazim Alsammak[4*]

[1,3,4]Department of Biology, College of Science, University of Mosul, Mosul, Iraq
[2]Department of Basic Sciences, College of Nursing, University of Mosul, Mosul, Iraq

Author Designation: [1,2]Lecturer, [3]Assistant Professor, [4]Professor

*Corresponding author: Essra Ghanim Hazim Alsammak (e-mail: esrsbio19@uomosul.edu.iq).

Abstract **Objectives:** *Enterobacter hormaechei*, a significant opportunistic pathogen, is associated with antimicrobial resistance including Urinary Tract Infections (UTIs). Limited genomic information is available from Iraqi clinical isolates of *E. hormaechei*. This research involved an investigation of the antimicrobial resistance genes and genomic-level determinants of a single *E. hormaechei* clinical isolate, recovered from a UTI patient in Mosul, Iraq. The isolate was initially identified through standard bacteriological testing and 16S rRNA gene sequencing. Whole-Genome Sequencing (WGS) was performed using the Illumina NovaSeq X platform after library preparation with the Illumina TruSeq Nano DNA Library Preparation Kit (350-bp insert size), generating 150-bp paired-end reads and the reads were assembled de novo using SPAdes. Genome annotation was performed using the RAST server, resistance genes were screened using CARD (v3.2.6) and phylogenomic placement was assessed using TYGS and MEGA-11. **Results:** The isolate, designated ESE.iq, was resistant to 13 of the 14 antibiotics tested, with meropenem remaining active. The isolate therefore met the XDR criterion based on the tested antimicrobial classes. The draft genome assembly comprised 63 contigs totaling 4,748,095 bp, with a G+C content of 55.25% and 4,544 Predicted Coding Sequences (CDSs). CARD identified 17 resistance-associated genes, including blaCTX-M-15, blaTEM-1B, blaOXA-1, qnrB1, fosA2, tet(A), sul2, dfrA14 and aac(6')-Ib-cr. Whole-genome phylogenomics placed ESE.iq closest to *E. hormaechei* subsp. xiangfangensis LMG 27195 (isDDH 93.8%). The BioProject accession is PRJNA1215288, whereas JBLHDI000000000 is the genome assembly accession. **Conclusion:** The ESE.iq draft genome contained diverse resistance-associated determinants that were consistent with the observed XDR phenotype. Genome-based analysis supported assignment of the isolate to *E. hormaechei* and identified multiple predicted resistance mechanisms. These isolate-specific findings provide genomic information from Mosul, Iraq and support continued surveillance of antimicrobial resistance in Enterobacter. Functional studies and comparative analyses of additional Iraqi isolates are needed to determine the broader epidemiological significance of these findings.

Key Words Enterobacter Hormaechei, Whole-Genome Sequencing, XDR, blaCTX-M-15, ESKAPE Pathogens

INTRODUCTION

UTIs are common clinical infections and antimicrobial resistance can complicate treatment and increase the risk of persistent or severe disease [1,2]. Although UPEC is the predominant uropathogen, *Enterobacter* species are also clinically relevant causes of UTI, particularly in healthcare-associated settings [3,4].

Complicated UTIs are more likely in patients with predisposing factors such as urinary tract abnormalities, long-term catheterization, immunosuppression, or recent antibiotic exposure. These factors can increase the likelihood of infection with antimicrobial-resistant *Enterobacter*.

Enterobacter spp. are Gram-negative bacilli that occur in human and animal intestinal microbiota and are also recovered from clinical specimens and hospital environments. Members of the genus are included among the ESKAPE pathogens and are associated with healthcare-associated infections and multidrug resistance [5,6].

The Enterobacter Cloacae Complex (ECC) comprises phenotypically similar but genetically distinct species, including *E. hormaechei*, *E. cloacae*, *E. kobei* and *E. ludwigii*.*E. hormaechei* is an important clinical member of the complex and has been associated with bloodstream, respiratory, urinary and other healthcare-associated infections [7,8].

Antimicrobial resistance in ECC results from both intrinsic and acquired mechanisms. Chromosomal AmpC β-lactamases contribute to intrinsic resistance to several β-lactam agents, while acquired ESBLs and carbapenemases can further expand the resistance phenotype [9,10].

E. hormaechei clinical isolates may carry resistance determinants affecting aminoglycosides, fluoroquinolones, cephalosporins, colistin, tigecycline and carbapenems. Resistance determinants can also disseminate through mobile genetic elements, increasing the potential for multidrug-resistant phenotypes [11,12].

Carbapenem-resistant *E. hormaechei* has been reported internationally, whereas genomic information from Iraqi clinical isolates, particularly from Mosul, remains limited. Characterizing an isolate from this setting can therefore contribute isolate-specific genomic data and help identify resistance determinants that warrant further surveillance.

This study aimed to characterize the genomic features and antimicrobial resistance gene profile of the single *E. hormaechei* isolate ESE.iq recovered from a urine specimen of a patient with UTI in Mosul, Iraq, using whole-genome sequencing and comparative genomic analysis.

METHODS

Bacterial Strain

An *Enterobacter hormaechei* isolate, designated ESE.iq, was obtained from a midstream urine specimen collected aseptically from a patient with a primary (first-episode) Urinary Tract Infection (UTI) at Al-Salam Teaching Hospital, Mosul, Iraq. The patient had no documented history of recurrent UTI. The urine specimen was processed using standard microbiological procedures and the isolate was included based on its characteristic growth and biochemical profile consistent with *E. hormaechei*. Preliminary identification was performed using conventional microbiological and biochemical tests, followed by molecular identification through amplification and sequencing of the 16S rRNA gene. The obtained sequence was compared with reference sequences available in the NCBI GenBank database for species-level identification.

Molecular Identification

DNA Extraction: Genomic DNA was extracted from a single colony using the Geneaid Biotech Genomic DNA Extraction Kit (Taiwan) according to the manufacturer's instructions.

DNA Purification and Quantification

DNA concentration and purity were assessed spectrophotometrically at 260 and 280 nm using a NanoDrop instrument (Cambridge CB4, England). The DNA integrity (DIN) and concertation for WGS was performed at Macrogen using an Agilent Technologies 2100 Bioanalyzer and QuantiFluor® dsDNA System accordingly. The DNA was passed with DIN >7 and a concertation of 54.05 ng/μL.

16S rRNA Gene Amplification (Polymerase Chain Reaction)

The 16S rRNA gene was amplified using the universal bacterial primers 27-F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1552-R (5′-AAGGAGGTGATCCARCCGCA-3′), purchased from Geneaid Biotech (Taiwan) [13]. PCR amplification was performed in a final reaction volume of 25 μL. The reaction mixture consisted of [12.5 μL] of 2× PCR Master Mix, [1.0 μL] of forward primer (10 pmol/μL), [1.0 μL] of reverse primer (10 pmol/μL), [2.0 μL] of template DNA and [8.5 μL] of nuclease-free water [13].

PCR amplification was performed using a thermal cycler under the following conditions: an initial denaturation at 95°C for 3 min, followed by [30-35] cycles of denaturation at 95°C for [30 s], annealing at [55°C] for [30 s] and extension at 72°C for [60-90 s]. A final extension was performed at 72°C for [7-10 min], followed by a hold at 4°C. The PCR products were separated by agarose gel electrophoresis to verify successful amplification. A single amplicon of approximately 1,500 bp was considered indicative of successful amplification of the nearly full-length 16S rRNA gene. The amplified PCR products were subsequently purified and subjected to sequencing for molecular identification of the bacterial isolates.

Sequence Analysis of 16S rRNA Amplicons

PCR products were submitted to Samogen Company (USA) for 16S rRNA gene sequencing. The PCR amplicon was approximately 1500 bp in length. Sequencing was performed using the forward primer and the resulting sequence was subjected to quality assessment to identify and remove low-quality and ambiguous nucleotide positions. The high-quality sequence obtained was approximately 1500 bp in length and was subsequently used for comparison with reference sequences in the NCBI GenBank database and for phylogenetic analysis.

Whole-Genome Sequencing

Illumina NovaSeq X was used as an insertion sequencing platform. The DNA library was prepared using Illumina TruSeq Nano DNA library with 350 bp insert size .The reads were generated with 150 bp paired-end reads.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility was assessed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar using the antimicrobial panel listed in Table 1. The susceptibility testing and interpretation were performed with reference to the Clinical and Laboratory Standards Institute (CLSI) M100, 35th edition (2025) [14]. Inhibition-zone diameters were measured in millimeters and interpreted according to the applicable CLSI zone-diameter breakpoints for Enterobacterales. The specific breakpoints used for each antimicrobial agent are presented in Table 1.

Genome Submission and Assembly

The draft genome assembly of *Enterobacter hormaechei* ESE.iq was submitted to the DDBJ, ENA and NCBI GenBank databases under genome assembly accession number JBLHDI000000000. The associated BioProject was registered under accession number PRJNA1215288.

Table 1: Antibiotics used for Antimicrobial Susceptibility Testing and CLSI Zone-Diameter Breakpoints

Antibiotics	Abbreviation	Concentration (µg/disk)	CLSI zone-diameter breakpoints (mm) S/I/R
Amoxicillin/Clavulanic acid	AMC	20	≥18/14-17/≤13
Cefazolin	CZ	30	≥23/20-22/≤19
Cefuroxime	CXM	30	≥18/15-17/≤14
Ceftazidime	CAZ	30	≥21/18-20/≤17
Ceftriaxone	CRO	30	≥23/20-22/≤19
Cefepime	FEP	30	≥25/19-24/≤18
Meropenem	MEM	10	≥23/20-22/≤19
Amikacin	AMK	30	≥20/17-19/≤16
Gentamicin	GEN	10	≥18/15-17/≤14
Ciprofloxacin	CIP	5	≥26/22-25/≤21
Ofloxacin	OFX	5	≥16/13-15/≤12
Fosfomycin	FOS	200	Not applicable*
Nitrofurantoin	NIT	300	≥17/15-16/≤14
Cotrimoxazole	SXT	25	≥16/11-15/≤10

*CLSI Disk-Diffusion Interpretive criteria for fosfomycin are restricted to urinary *Escherichia coli* isolates and were therefore not applied to *Enterobacter hormaechei* ESE.iq

Raw-Read Processing

Raw sequencing reads were processed to remove adapter sequences and low-quality reads using Trimmomatic version 0.36 [15]. A sliding-window approach with a minimum quality threshold of Q20 was applied and reads failing the quality criteria were removed prior to genome assembly.

Draft Genome Assembly

The quality-filtered reads were assembled de novo using SPAdes version 3.5 [16]. The assembly was performed using k-mer sizes of 21, 33, 55 and 77, while all other parameters were maintained at their default settings. The quality of the resulting draft genome assembly was assessed using QUAST to evaluate the assembly statistics and overall quality.

Whole Genome Based Phylogenetic Tree

Whole-genome phylogenetic analysis was performed using the TYGS platform [17]. The ESE.iq genome in FASTA format was submitted to the TYGS server. TYGS automatically determined closest neighbors type strains based on genome wide similarity of pairwise digital DNA-DNA hybridization (dDDH) values. The final tree was produced with FastME 2.0 [18], a distance-based algorithm built into the TYGS pipeline using 100 pseudo-bootstrap replicates.

In Silico DNA-DNA (isDDH) Hybridization Analysis

Digital DNA-DNA Hybridization (isDDH) between ESE.iq and each reference type strain was calculated via the GGDH server [18], using whole-genome sequence data as input for each pairwise computation.

16S rRNA Gene Phylogenetic Analysis

The 16S rRNA sequence of ESE.iq was used as a BLASTn query against the NCBI GenBank nucleotide database to retrieve homologous sequences. A Neighbor-Joining tree was constructed in MEGA-11 [19] and branch support was assessed using 1000 bootstrap replicates.

Detection of the Antibiotic Resistance Genes in the Genome of *Enterobacter hormaechei* ESE.iq

Resistance-associated determinants in the draft genome of *Enterobacter hormaechei* ESE.iq, including antimicrobial resistance genes and genes associated with biocide and antiseptic tolerance, were identified using the Comprehensive Antibiotic Resistance Database (CARD) version 3.2.6 [20]. We performed an analysis of the assembled genome sequence using the CARD tool with the search restricted to Perfect and Strict hits and a minimum sequence identity threshold of ≥90%. The identified resistance determinants were subsequently recorded and used for characterization of the antimicrobial resistance profile of ESE.iq.

Genome-Level Comparison

Genome-level synteny and comparative genomic relationships of E. hormaechei ESE.iq were visualized using the BLAST Ring Image Generator (BRIG) [21]. The draft genome of ESE.iq was compared with eleven reference genomes and the resulting similarities were represented as concentric rings. The intensity of the color at each genomic position indicates the degree of nucleotide sequence identity between ESE.iq and the corresponding reference genome, allowing visualization of conserved and variable genomic regions.

RESULTS

The isolate recovered from the urine specimen of a patient with UTI was designated ESE.iq. Preliminary identification by conventional microbiological methods and 16S rRNA sequencing supported assignment to the genus *Enterobacter*. Whole-genome-based taxonomic analysis using TYGS provided the principal evidence for species-level assignment as *E. hormaechei*, with the isolate placed closest to *E. hormaechei* subsp. xiangfangensis. The genome assembly was deposited under accession JBLHDI000000000 and the associated BioProject was registered under PRJNA1215288.

Antibiotic Susceptibility Testing

The antimicrobial resistance profile of *E. hormaechei* ESE.iq is shown in Table 2. The table reports the categorical interpretations obtained by disc diffusion testing; inhibition-zone diameters are not shown in the current version.

Disc diffusion testing classified *E. hormaechei* ESE.iq as resistant to 13 of the 14 agents tested, with meropenem remaining susceptible. Based on the tested antimicrobial classes, the isolate met the XDR criterion under the international expert consensus definition [22].

The observed phenotype should be interpreted as specific to this isolate and the antimicrobial panel used. Similar multidrug-resistant and XDR phenotypes have been reported among clinical *E. hormaechei* collections, including isolates carrying multiple β-lactam, quinolone, aminoglycoside, sulfonamide, tetracycline and fosfomycin resistance determinants [23,24].

A previous study reported resistance among *E. hormaechei* isolates to nitrofurantoin (100%), ceftazidime and cotrimoxazole (60% each), gentamicin and ciprofloxacin (40% each) and amikacin (20%), whereas meropenem remained active [25].

Dehkordi *et al.* [1] reported resistance among *E. hormaechei* isolates to nitrofurantoin, imipenem, ciprofloxacin, gentamicin, amikacin and cotrimoxazole, with reported resistance proportions of 23.1, 30.8, 46.1, 53.8, 69.2 and 84.6%, respectively.

Table 2: Antibiotics Resistance Profile of *Enterobacter hormaechei* Strain ESE.iq

Antibiotics	*Enterobacter hormaechei* strain ESE.iq
Amoxicillin/Clavulanic acid	R
Cefazolin	R
Cefuroxime	R
Ceftazidime	R
Ceftriaxone	R
Cefepime	R
Meropenem	S
Amikacin	R
Gentamicin	R
Ciprofloxacin	R
Ofloxacin	R
Fosfomycin	R
Nitrofurantoin	R
Cotrimoxazole	R

S: Sensitive, R: Resistant

Seriki *et al.* [23] described a broadly comparable resistance pattern in *E. hormaechei* AH1-NIMR, which was resistant to ten of twelve agents evaluated: Amoxicillin /Clavulanic acid, Cefuroxime, Ceftazidime, Cefepime, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Cotrimoxazole and Aztreonam. Chloramphenicol and the third-generation cephalosporin cefotaxime remained active.

Draft Genome Features of *Enterobacter hormaechei* Strain ESE.iq

The draft genome assembly of *E. hormaechei* ESE.iq (accession JBLHDI000000000) comprised 63 contigs totaling 4,748,095 bp, with a G+C content of 55.25%. Contig sizes ranged from 521 to 385,990 bp, with an N50 of 242,003 bp. Annotation identified 4,544 predicted CDSs and 65 tRNA genes, together with rRNA genes comprising 1 copy of 5S rRNA, 4 copies of 16S rRNA and 3 copies of 23S rRNA as in Table 3.

RAST annotation assigned genes to multiple subsystem categories. The largest categories were amino acids and derivatives (346 genes), carbohydrates (319), protein metabolism (219), cofactors/vitamins/prosthetic groups/pigments (156), virulence/disease/defense (54) and cell division/cell cycle (7). These categories provide a functional overview of the draft genome and are relevant to interpreting its metabolic capacity and potential defense-associated functions. The complete distribution is shown in Figure 1.

Whole-genome phylogenomics using TYGS (Figure 2; Table 4) placed ESE.iq within *E. hormaechei*. The closest type-strain genome was *E. hormaechei* subsp. xiangfangensis LMG 27195 (isDDH 93.8%), followed by *E. hormaechei* subsp. oharae DSM 16687 (75.7%) and subsp. steigerwaltii DSM 16691 (75.6%). These genome-based results provide the principal evidence for the species-level assignment.

Table 3: Genomic Assembly and Annotation Metrics for *E. hormaechei* ESE.iq, Computed with QUAST and the RAST Platform [26]

Feature	Value
Genome total length (bp)	4,748,095
Number of contigs	63
Largest contig (bp)	385,990
Smallest contig (bp)	521
GC content (%)	55.25
Total of protein-Coding Sequences (CDSs)	4,544
Number of tRNA genes	65
rRNA genes (5S/16S/23S)	1/4/3
N50	242,003
Sequencing depth/coverage	the mean sequencing depth is approximately 72×
Contamination assessment	Genome contamination = 0.66%

Table 4: Genome Pairwise Comparisons of Enterobacter hormaechei ESE.iq Genome Vs Type Strain Genomes based on isDDH, GC Content, δ- Value, Genome Size and Number of Proteins

Enterobacter hormaechei ESE.iq vs. type strain genomes	Digital isDDH value (%)	Percent G+C (%)	δ- value	Genome Size (bp)	No. of proteins
Enterobacter hormaechei subsp. *xiangfangensis* LMG 27195	93.8	55.25	0.062	4,661,849	4504
Enterobacter hormaechei subsp. *oharae* DSM 16687	75.7	55.58	0.076	4,724,316	4436
Enterobacter hormaechei subsp. *steigerwaltii* DSM 16691	75.6	55.55	0.066	4,782,480	4424
Enterobacter hormaechei subsp. *hoffmannii* DSM 14563	65.6	55.33	0.099	4,678,566	4321
Enterobacter hormaechei ATCC 49162	59.9	55.24	0.111	4,802,284	4671
Enterobacter pasteurii A-8	43.7	56.41	0.053	4,810,455	4376
Enterobacter bugandensis EB-247	35.6	56.1	0.052	4,717,613	4332
Enterobacter nematophilus E-TC7	35.2	56.36	0.051	4,707,174	4293
Enterobacter roggenkampii DSM 16690	35	55.5	0.076	4,919,759	4594
Enterobacter tabaci KCTC 4269	34.4	56.04	0.066	4,899,997	4474

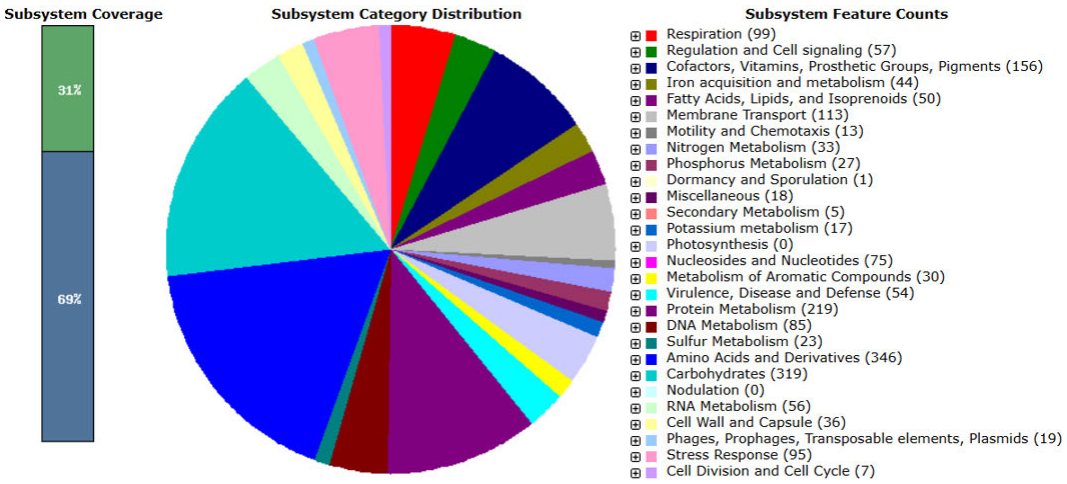

Figure 1: RAST Subsystem-Category Distribution for *E. hormaechei* ESE.iq, Visualized with the Seed Viewer. The Figure Summarizes the Distribution of Annotated Genes among Major Functional Subsystems and is Included to Provide Context for the Genomic Features Relevant to metabolism, Cellular Processes and Defense

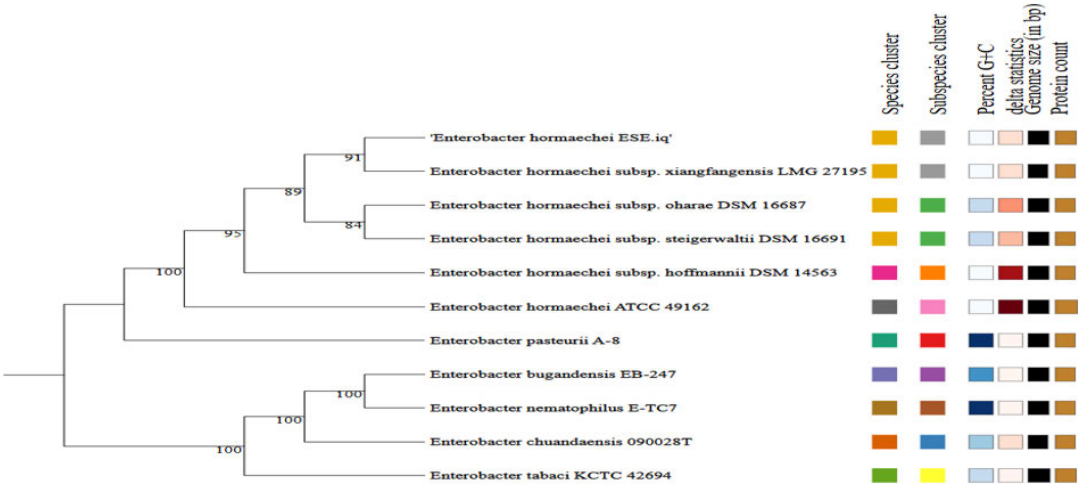

Figure 2: TYGS Whole-Genome Phylogenetic Tree for *E. hormaechei* ESE.iq. The Topology Was Generated With FastME 2.0; 100 Pseudo-Bootstrap Replicates were used for Branch-Support Assessment. Each Leaf is Labelled with the Corresponding Taxonomic Cluster and Available Genomic Metrics. Ese.iq should be Clearly Identified in the Final Figure, and the Scale Bar should be Retained

The isDDH value of 93.8% between ESE.iq and LMG 27195 exceeds the commonly applied 70% species-delineation threshold and supports assignment to *E. hormaechei*. The isolate was most closely related to subsp. xiangfangensis; however, subspecies-level interpretation should be based primarily on genome-wide evidence rather than 16S rRNA similarity alone.

The 16S rRNA Neighbor-Joining tree (MEGA-11; scale bar 0.002) showed ESE.iq clustering with E. hormaechei subsp. oharae DSM 16687 and subsp. xiangfangensis LMG 27195, with 99.72% sequence identity to both. These similarities indicate close phylogenetic relatedness, but they are not, by themselves, sufficient for definitive species or subspecies assignment. The genome-based TYGS analysis therefore remains the principal basis for taxonomic interpretation as in Figure 3 and Table 5.

BRIG alignment of the ESE.iq draft genome against eleven reference strains (Figure 4) was broadly consistent with the isDDH results. Strong synteny was observed with *E. hormaechei* subsp. xiangfangensis LMG 27195 and subsp. oharae DSM 16687. Gaps in the rings indicate regions that are not shared between the compared genomes; these regions may represent accessory or strain-specific sequence, but their association with antimicrobial-resistance or virulence genes cannot be inferred from the BRIG plot alone.

CARD analysis identified 17 resistance-associated genes in the ESE.iq draft genome (Table 6). The detected genes were broadly consistent with several components of the observed resistance phenotype. However, gene detection represents a bioinformatics prediction and does not demonstrate gene expression or establish a causal relationship with the phenotype.

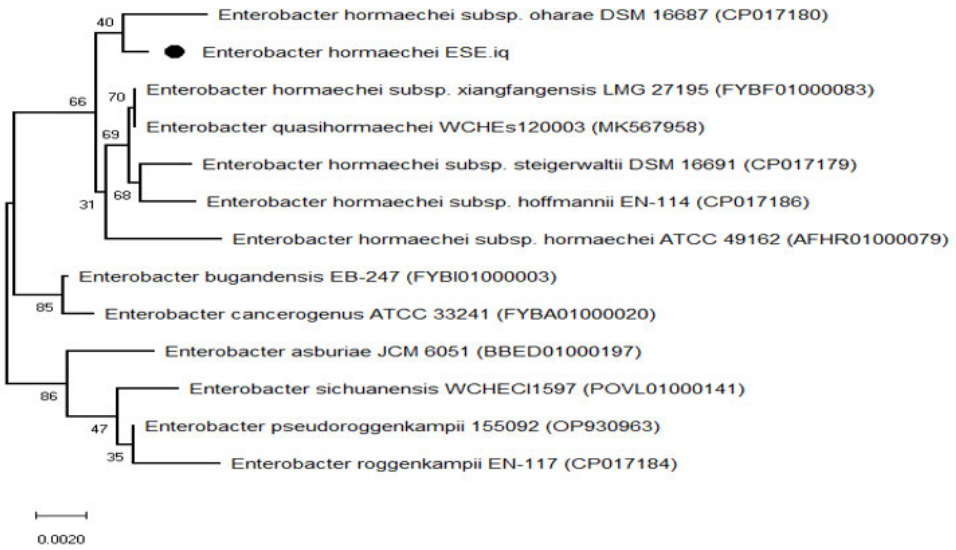

Figure 3: Neighbor-Joining tree generated in MEGA-11 (scale bar 0.002) showing the relationship of *E. hormaechei* ESE.iq to closely related sequences based on 16S rRNA. Numbers at the nodes indicate the percentage of 1000 bootstrap replicates supporting the corresponding clades. ESE.iq should be clearly labelled and the scale bar and bootstrap values should remain readable

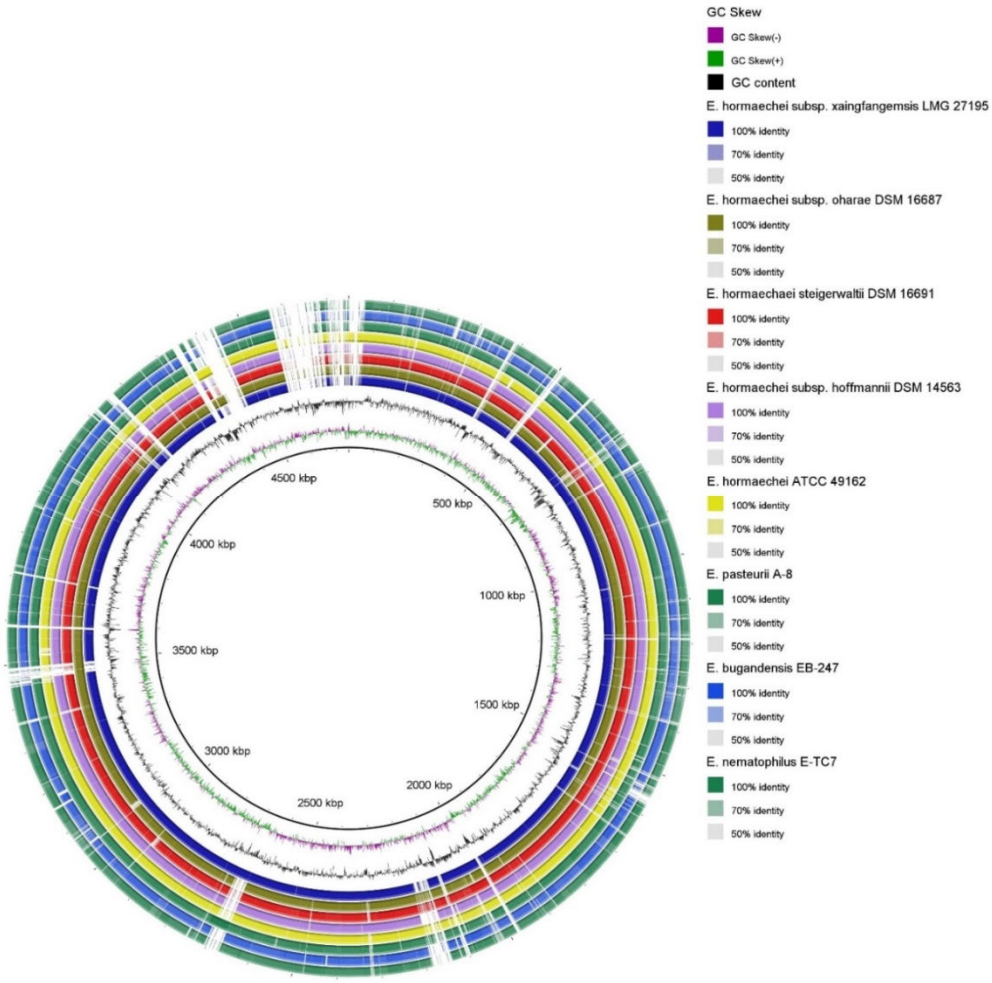

Figure 4: BRIG plot comparing the *E. hormaechei* ESE.iq draft genome with eleven reference genomes. The inner rings show GC skew and GC content, while each outer ring represents a reference genome. Ring gaps indicate regions absent from the corresponding comparison and should not be interpreted as resistance or virulence islands without additional genomic analysis

Table 5: Most Closely Related Bacterial Species to *E. hormaechei* ESE.iq, Identified by 16S rRNA BLASTn Search in GenBank, with Corresponding Accession Numbers and Percent Sequence Identity

Species name	Strain name	Accession No.	Similarity (%)
Enterobacter hormaechei subsp. *oharae*	DSM 16687	CP017180	99.72
Enterobacter hormaechei subsp. *xiangfangensis*	LMG 27195	FYBF01000083	99.72
Enterobacter quasihormaechei	WCHEs120003	MK567958	99.57
Enterobacter hormaechei subsp. *steigerwaltii*	DSM 16691	CP017179	99.43
Enterobacter hormaechei subsp. *hoffmannii*	EN-114	CP017186	99.43
Enterobacter hormaechei subsp. *hormaechei*	ATCC 49162	AFHR01000079	99.15
Enterobacter bugandensis	EB-247	FYBI01000003	99.14
Enterobacter cancerogenus	ATCC 33241	FYBA01000020	99.14
Enterobacter asburiae	JCM 6051	BBED01000197	99.12
Enterobacter pseudoroggenkampii	155092	OP930963	99.0
Enterobacter sichuanensis	WCHECl1597	POVL01000141	98.8
Enterobacter roggenkampii	EN-117	CP017184	98.6

Table 6: Antimicrobial Resistance-Associated Genes Detected in *E. hormaechei* ESE.iq using CARD (v3.2.6). The Predicted Phenotype Column Represents a Bioinformatic Prediction and does not Demonstrate Gene Expression or Phenotypic Resistance

Resistance gene	Drug class	Resistance mechanism	Predicted phenotype (bioinformatic)	Percentage identity
blaOXA-1	cephalosporin, penicillin beta-lactam	antibiotic inactivation	oxacillin, amoxicillin, piperacillin, ampicillin, cefalotin	100
blaTEM-1B	monobactam, cephalosporin, penicillin beta-lactam	antibiotic inactivation	cefazolin, amoxicillin, ampicillin, cefalotin	100
blaCTX-M-15	cephalosporin, penicillin beta-lactam	antibiotic inactivation	cefazolin, ceftazidime, ceftriaxone, ampicillin, cefalotin	100
dfrA14	diaminopyrimidine antibiotic	antibiotic target replacement	trimethoprim	100
sul2	sulfonamide antibiotic	antibiotic target replacement	sulfadiazine, sulfadimidine, sulfadoxine, sulfamethoxazole, sulfisoxazole, sulfacetamide, mafenide, sulfasalazine, sulfamethizole	100
QnrB1	fluoroquinolone antibiotic	antibiotic target protection	ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin, nalidixic acid, norfloxacin, sparfloxacin	100
catB3	phenicol antibiotic	antibiotic inactivation	chloramphenicol	100
aph(6)-Id	aminoglycoside antibiotic	antibiotic inactivation	streptomycin	99.64
aadA1	aminoglycoside antibiotic	antibiotic inactivation	spectinomycin, streptomycin	99.61
aph(3")-Ib	aminoglycoside antibiotic	antibiotic inactivation	dibekacin, sisomicin, netilmicin, tobramycin, 6'-N-ethylnetilmicin, 2'-N-ethylnetilmicin, gentamicin	98.95
aac(6')-Ib-cr	aminoglycoside antibiotic	antibiotic inactivation	amikacin, kanamycin A, tobramycin	97.98
FosA2	phosphonic acid antibiotic	antibiotic inactivation	fosfomycin	97.16
msbA	nitroimidazole antibiotic	antibiotic efflux	metronidazole	94.85
tet(A)	tetracycline antibiotic	antibiotic efflux	tetracycline	94.36
emrB	fluoroquinolone antibiotic	antibiotic efflux	nalidixic acid	93.79
rsmA	fluoroquinolone antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic	antibiotic efflux	trimethoprim, chloramphenicol	85.25
leuO	nucleoside antibiotic, disinfecting agents and antiseptics	antibiotic efflux	acriflavine, puromycin	81.85

The β-lactamase genes blaOXA-1, blaTEM-1B and blaCTX-M-15, together with aminoglycoside-modifying genes aph(6)-Id, aadA1, aph(3")-Ib and aac(6')-Ib-cr, were among the detected determinants. The latter may also contribute to reduced fluoroquinolone susceptibility. Additional determinants included emrB, qnrB1, dfrA14, sul2, fosA2, tet(A), msbA, catB3, rsmA and leuO.

The detected resistance-associated genes were assigned to four predicted mechanisms: Antibiotic inactivation, target protection, target replacement and efflux. These assignments should be interpreted as CARD-based bioinformatic predictions rather than direct experimental demonstrations of mechanism.

DISCUSSION

Recurrent urinary tract infections are clinically important because antimicrobial resistance can complicate treatment and contribute to persistent infection. Accurate organism identification and antimicrobial susceptibility testing remain essential for guiding therapy. In the present study, whole-genome analysis was used to characterize a single *E. hormaechei* clinical isolate and to investigate its resistance-associated genomic features.

E. hormaechei is an important opportunistic pathogen in healthcare settings and species-level identification within the *E. cloacae* complex can be challenging [11,8]. In this study, 16S rRNA sequencing provided preliminary identification, whereas whole-genome phylogenomics using TYGS provided the principal genome-based evidence supporting assignment of ESE.iq to *E. hormaechei*. The resistance profile showed broad phenotypic resistance and was generally concordant with several resistance-associated genes detected by CARD.

Molecular Identification and Phylogenetic Placement

The isolate recovered from a urine specimen from a patient with UTI in Mosul was designated *E. hormaechei* ESE.iq. Although 16S rRNA analysis showed 99.72% similarity to two *E. hormaechei* subspecies, these values indicate close relatedness rather than definitive subspecies assignment. Whole-genome analysis using TYGS yielded an isDDH value of 93.8% with *E. hormaechei* subsp. xiangfangensis LMG 27195, providing the principal genomic support for species-level assignment and close relationship to this subspecies. Comparative studies of *E. hormaechei* and the *E. cloacae* complex likewise support the use of genome-wide analyses to resolve relationships that may not be fully resolved by 16S rRNA alone [24].

The 63 contig ESE.iq draft genome assembly was 4,748,095 bp in length with a G+C content of 55.25% and an N50 of 242,003 bp. The assembly consists of 4544 predicted CDSs, 65 tRNA genes, 1 copy of 5S rRNA and 4 and 3 copies of 16S and 23S rRNA respectively. Comparative analysis using BRIG showed considerable synteny with closely related *E. hormaechei* genomes. The non-shared regions that we observed may be accessory sequence, but their function cannot be determined from BRIG alone.

Antibiotic Susceptibility Profile

Disc diffusion testing classified *E. hormaechei* ESE.iq as resistant to 13 of the 14 agents tested, while meropenem remained susceptible. The resistance profile included β-lactams, aminoglycosides, fluoroquinolones, fosfomycin, nitrofurantoin and cotrimoxazole. The isolate met the XDR criterion under the international consensus framework based on the tested antimicrobial classes. Because this study concerns a single isolate, the resistance profile should not be generalized to *E. hormaechei* populations in Iraq. Similar multidrug-resistant and XDR phenotypes have been reported among clinical *E. hormaechei* collections, including isolates carrying multiple β-lactam, quinolone, aminoglycoside, sulfonamide, tetracycline and fosfomycin resistance determinants [23,24].

ESE.iq remained susceptible to meropenem. The absence of major acquired carbapenemase genes, including blaKPC, blaNDM and blaOXA-48-like genes, is consistent with this phenotype, but it does not establish the cause of susceptibility. Carbapenem activity may also be influenced by porin expression, efflux, AmpC/ESBL activity and other mechanisms; therefore, the genomic findings should be interpreted cautiously. The retained meropenem susceptibility should therefore be interpreted cautiously: in *Enterobacter cloacae* complex, carbapenem susceptibility can be influenced by the combined effects of carbapenemases, AmpC expression, outer-membrane permeability and efflux rather than by carbapenemase detection alone [26-28].

Genomic Resistance Mechanisms

CARD analysis identified 17 resistance-associated genes in the ESE.iq draft genome, representing four predicted mechanisms: antibiotic inactivation, target protection, target replacement and efflux. The presence of these genes indicates potential resistance determinants but does not demonstrate expression or direct phenotypic causality.

β-Lactam Resistance

The β-lactamase genes detected in ESE.iq-blaCTX-M-15, blaTEM-1B and blaOXA-1-are associated with β-lactam resistance. CTX-M-15 is an ESBL associated with resistance to several third-generation cephalosporins, whereas OXA-1 and TEM-1B predominantly have penicillinase activity and may contribute to resistance to penicillin derivatives and some early-generation cephalosporins. The coexistence of these genes provides a potential genetic basis for the observed β-lactam resistance, but functional expression was not assessed in this study. A recent genomic study of *E. hormaechei* reported the same combination of blaCTX-M-15, blaTEM-1B and blaOXA-1 together with additional aminoglycoside and quinolone resistance determinants, supporting the plausibility of this resistance-gene constellation in *E. hormaechei* [29].

Aminoglycoside and Quinolone Resistance

Resistance-associated aminoglycoside-modifying genes detected in ESE.iq included aph(6)-Id, aadA1, aph(3")-Ib and aac(6')-Ib-cr. The aac(6')-Ib-cr determinant is notable because it is associated with modification of certain aminoglycosides and reduced susceptibility to selected fluoroquinolones. The plasmid-mediated qnrB1 gene is associated with quinolone target protection. Together, these determinants may contribute to the observed aminoglycoside and fluoroquinolone resistance phenotype, but their individual contributions were not experimentally validated.

Efflux-Mediated and Multi-Class Resistance

Four efflux-associated genes, msbA, tet(A), emrB and rsmA, were detected in the ESE.iq genome, together with leuO, a regulatory gene associated with modulation of multidrug efflux systems. These genes are predicted to contribute to efflux-mediated resistance, but the present study did not experimentally assess efflux activity or gene expression. The lower sequence identities observed for rsmA (85.25%) and leuO (81.85%) warrant particular caution in interpreting these hits as functional resistance determinants.

Additional resistance-associated determinants included fosA2, catB3, sul2 and dfrA14. These genes are predicted to be associated with fosfomycin, chloramphenicol, sulfonamide and trimethoprim resistance, respectively. Because chloramphenicol and tetracycline were not included in the antimicrobial susceptibility panel, the corresponding genotype-phenotype relationships could not be phenotypically assessed in this study. Thus, these findings should be regarded as bioinformatic predictions rather than demonstrated resistance. Because these genes were not all represented in the susceptibility panel, their presence should remain an in-silico prediction rather than a demonstrated phenotype, consistent with the limitations of genotype-phenotype inference in WGS-based AMR surveillance [24,8].

Clinical and Epidemiological Implications

β-lactamase-producing multidrug-resistant Gram-negative bacteria are clinically important because they can resist multiple antimicrobial classes and limit treatment options. Mobile genetic elements, including conjugative plasmids, can facilitate dissemination of resistance determinants among *Enterobacter*ales and other opportunistic pathogens [30,31]. In the present study, these broader mechanisms provide context for interpreting the resistance-associated genes detected in the single ESE.iq isolate, but the study does not establish transmission or dissemination of these determinants.

The identification of XDR *E. hormaechei* ESE.iq from a UTI in Mosul provides isolate-specific genomic and antimicrobial-resistance information from a region with limited published data. The coexistence of β-lactamase genes, aminoglycoside-modifying determinants, efflux-associated genes and other resistance-associated genes may contribute to the broad resistance phenotype observed in this isolate. Meropenem susceptibility was retained, but its clinical significance should be interpreted for this isolate only and in conjunction with phenotypic susceptibility testing. Recent genomic surveillance also demonstrates that *E. hormaechei* can carry multiple resistance determinants across β-lactam, aminoglycoside, quinolone, sulfonamide, tetracycline and fosfomycin classes, reinforcing the value of continued genomic surveillance while not establishing transmission from a single isolate [24,29].

For this isolate, the combination of phenotypic susceptibility testing and genome-based analysis provided complementary information on antimicrobial resistance. WGS also supported genome-based taxonomic placement and identification of resistance-associated determinants. However, the present data do not establish the expression or clinical effect of individual resistance genes and the findings should not be generalized to all *E. hormaechei* isolates in Iraq. This complementary interpretation is consistent with the broader use of WGS to characterize resistance determinants and phylogenetic relationships in *E. hormaeche* [24,24].

Study Limitations and Future Perspectives

This investigation was limited to a single clinical isolate, which restricts generalization to the broader Iraqi *E. hormaechei* population. Additional limitations include the absence of functional validation of resistance genes, lack of detailed plasmid/mobile-element characterization, the limited antimicrobial susceptibility panel and the absence of comparative Iraqi clinical isolates. Future multicenter studies should include additional isolates and functional analyses to assess the expression, contribution and epidemiological distribution of resistance determinants, particularly the lower-identity hits such as rsmA (85.25%) and leuO (81.85%). The absence of functional validation and plasmid/mobile-element characterization also limits causal inference from resistance-gene detection [24,27].

CONCLUSION

E. hormaechei ESE.iq exhibited a diverse repertoire of resistance-associated genes that was broadly consistent with its observed XDR phenotype. Whole-genome analysis supported genome-based species assignment, comparative phylogenomic placement and identification of predicted resistance determinants. These findings provide isolate-specific genomic data from Mosul, Iraq. Broader conclusions about *E. hormaechei* in Iraq require additional comparative isolates, functional validation and epidemiological surveillance.

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Consent for Publication

Not applicable. The manuscript does not contain any patient-identifiable information, images, or personal clinical data.

Availability of Data and Materials

The assembled genome sequence generated during the current study is available in the DDBJ/ENA/GenBank databases under accession number JBLHDI000000000 and BioProject accession number PRJNA1215288. The raw sequencing reads are available in the NCBI Sequence Read Archive under accession number SRR40226449, with the associated BioSample accession number SAMN46400533.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

Sahar Salim Petrus Al-Nakkar: Writing-original draft preparation, formatting and supervision of data collection. **Amerah Ali Ahmed:** Data curation, investigation and validation. **Enas Abdul Munieem Al-Layla:** Software, resources and visualization. **Essra Ghanim Al-Sammak:** Conceptualization, methodology, supervision, project administration and writing-review and editing. All authors read and approved the final manuscript.

Ethical Statement

Institutional ethics approval was obtained from the Research Ethics Committee of the University of Mosul, Mosul, Iraq (Approval No. 2686; Date:16/05/2023). Written informed consent was waived by the ethics committee because the study involved only the use of de-identified bacterial isolates and did not include any patient-identifiable information, clinical data, or direct patient contact. All methods were performed in accordance with the relevant institutional guidelines and regulations.

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