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التنميط الجيني لعوامل الضراوة وتنوع المجموعات المصلية في الإشريكية القولونية المرتبطة بتسمم الدم.

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المستخلص :

تُعدّ الإشريكية القولونية سببًا رئيسيًا لعدوى مجرى الدم والإنتان على مستوى العالم، إلا أن الأبحاث التي تناولت العلاقة بين خصائص ضراوة البكتيريا والنتائج السريرية في دول الشرق الأوسط قليلة. في هذه الدراسة، تم تحديد المجموعات المصلية O وجينات عوامل الضراوة في عزلات الإشريكية القولونية من حالات الإنتان، وربطها بشدة الإنتان ومآل المرضى. تم الحصول على ستة وثمانين عزلة غير متكررة من الإشريكية القولونية من مزارع الدم لحالات الإنتان، والتي تراوحت أعمارها بين ٢٠ و٧٠ عامًا. تم الكشف عن الجينات المُحللة للدم باستخدام تفاعل البوليميراز المتسلسل المتعدد، وصُنفت المجموعات المصلية، وخضعت العوامل السريرية والميكروبيولوجية المرتبطة بشدة المرض والوفيات لتحليل إحصائي. ارتبطت الأنماط الجينية عالية الضراوة، التي تحمل جينات السموم وجينات مقاومة المصل، بالإضافة إلى المجموعات المصلية O السائدة (O25/O6/O15) وأنماط مقاومة الأدوية المتعددة، ارتباطًا وثيقًا بالإنتان الشديد والوفيات داخل المستشفى. تُبرز هذه البيانات دور التنميط الجزيئي للضراوة في اتخاذ القرارات السريرية، وتُشير إلى إمكانية استخدامه كأداة لتصنيف المخاطر وإدارة المضادات الحيوية في حالات الإنتان الناجم عن الإشريكية القولونية.

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الكلمات المفتاحية: الإشريكية القولونية، الإنتان، عدوى مجرى الدم، عوامل الضراوة، المجموعات المصلية O.

Genotypic profiling of virulence factors and serogroup variability in septicemia-associated *Escherichia coli*

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Abstract

Escherichia coli (E.coli) is a leading cause of bloodstream infection (BSI) and sepsis globally. For *E. coli*, the O-serogroups and VF genes in septicaemia isolates were determined and correlated with the severity of sepsis and outcome among patients. Eighty-six non-repetitive *E. coli* isolates were obtained from blood cultures of the septic cases, who were aged between 20 and 70 years. Haemolytic genes were detected by multiplex PCR, serogroups were classified, and the clinical and microbiologic factors related to the severity of illness and mortality were statistically analysed. High virulence genotypes having toxin genes, serum resistance genes, predominant dominant O-serogroups (O25/O6/O15), and multidrug resistant profiles were significantly associated with severe sepsis and in-hospital mortality. In this report, we add to the contribution of high-virulence genotypes (≥4 virulence genes, including toxin and complement resistance determinant) for *E. coli* septicaemia and predominant O-serogroups (O25/O6/O15) as independent predictors of severe clinical outcome, as well as in-hospital death rates, even more than multi-resistance phenotypes. These data provide evidence for integrating molecular virulence profiling in clinical risk stratification and antimicrobial stewardship programmes,

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particularly in resource-limited settings where focused intervention scales more effectively to impact outcomes.

Keywords: *Escherichia coli*, Sepsis, Bloodstream infection, Virulence factors, O-serogroups

1- Introduction:

Sepsis remains one of the leading causes of morbidity and mortality worldwide, particularly in low- and middle-income countries (LMICs), where weakened clinical infrastructures, poor diagnostic capacity, antimicrobial resistance, and lack of infection control measures compound this challenge. *E. coli* is the most frequent Gram-negative pathogen isolated from bloodstream infections (BSIs) and a significant aetiology of community- and hospital-acquired sepsis (Mun, Seok Jun, et al., 2022, p. 336)- (Bandy et al., 2020, p. e0233704). In resource-limited settings such as Iraq, the case fatality rates for *E. coli*-associated sepsis are reported to be up 30% and underscore a clear need for better risk stratification and targeted therapeutic interventions (Hasan et al., 2024, p. 33-39).

The natural history of *E. coli* sepsis and outcome is influenced by host-related but also pathogen-related factors, including age, co-morbid status, and immune state. However, the inherent virulence capacity of the infecting strain contributes further to the establishment of the clinical profile. Isolates of ExPEC present many VFs that can facilitate systemic invasion and immune evasion. These include adhesins (*fimH, papC*) that mediate attachment to the surface of host epithelial cells; iron acquisition systems (*iutA, fyuA*), used as defence in a host deprived of iron, like serum; and determinants for resistance to killing by serum (*iss*, *traT*), protecting from complement-mediated lysis and toxins (*hlyA, cnf1*), leading to tissue damage and an inflammatory response out of control (Kolenda, Rafał, et al., 2021, p. 000743.)- (Ahmadi et al., 2024, p. 15). The concurrent presence of more than one VF flank's high-virulence genotypes, which were increasingly

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linked to worse invasive infection sequelae (Zbinden et al., 2025, p. e00048-25).

Determination of serogroup based on O-antigen diversity also provides epidemiologic content. Some O-serogroups, such as O25, O6, and O15, are over-represented in invasive ExPEC isolates worldwide and are commonly associated with hypervirulence and multidrug resistance, particularly the pandemic clonal drug-resistant lineage of O25b-ST131 (Denamur, Erick, et al., 2021, p. 37-54). However, little is known about the distribution of these serogroups and their relationship with the VF behavior in sepsis reported from the Middle East region, where apparently local clonal dynamics may be influenced by distinct ecological factors such as high antibiotic pressure and decreased molecular monitoring.

In addition, environmental pollution (biomedical and industrial pollution) contributes to aggravating the public health risks in war-torn regions. Organic and metal–organic pollutants originating from laboratory and industry wastewater are presented as a significant international environmental problem that affects biodiversity and human health; for this reason, the necessity of removing organic compounds before discharge is emphasised (Jawad, Thaqeef M., et al., 2021, p. 4857-4865). The general situation of environmental degradation and antimicrobial contamination in the environment may remotely affect pathogens' evolution and resistance spread, even in, e.g., Iraq.

However, the local studies carried out in Iraq are much more focused on only antimicrobial susceptibility profiles than on proving bacterial virulence factors and clinical inference. This paucity has impeded the advancement of more targeted therapies for sepsis. To describe the association between virulence and disease severity in bacteremia patients, we conducted a WGS analysis of 86 nonrepetitive *E. coli* isolates isolated from adult septicaemic patients, as well as other relevant patient-related complications, giving O-serogrouping, multiplex PC paint-based identification of VFs, and detailed clinical data within five tertiary hospitals in Baghdad to quantify the relative

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contributions that bacterial virulence made toward worse outcomes represented here either by severity of illness or risk for death.

2- Materials and Methods:

2-1 Isolates Collection and Preservation:

A total of 86 on-duplicate *E. coli* isolates were prospectively collected from the blood cultures of adult patients (age range: 20–70 years) who were diagnosed with septicaemia in five Iraqi tertiary care hospitals located in Baghdad, Iraq, during the period between January 12th, 2025, and January 6th, 2026. The blood sample is collected using aseptic techniques for microbiological praxis, and 10 mL was inoculated directly into BACTEC™ Plus Aerobic/F culture bottles (Becton Dickinson, USA) from each patient. Bottles were incubated at 37°C in the BACTEC blood culture system (BACTEC FX™) and monitored for microbial growth, which was identified on the day of detection between days 1 and 5 after the start of incubation.

Positive blood culture bottles were subcultured on 5% sheep blood agar and MacConkey agar plates, which were then incubated aerobically at 37°C for 18–24 hours. Colonies with putative *E. coli* morphology were selected from colony characteristics, Gram staining, and simple biochemical tests (indole production, citrate utilisation, lactose fermentation , and motility). The isolates were confirmed to be *E. coli* on the basis of standard phenotypic characteristics according to CLSI guidelines. No duplicates/repeats from the same person were included, and only the first isolate per patient was considered to avoid duplication.

Primary patients with a gastrointestinal source of infection, asymptomatic bacteriuria, and non-septicaemic bloodstream colonisation were excluded. We selected ages 20–70 years to optimize coverage of adult septicaemia and, at the same time, not include patients (<20 years) nor subjects (as in ages over 70 years old), subjects exposed to immunosenescence or multimorbidity that would introduce confounding not directly related to the

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virulence of the microorganism involved. This figure is in line with extensive local reports of adult sepsis from Iraq and the neighbouring areas.

Positive isolates were stored at −20°C in tryptic soy broth with 20% (v/v) glycerol for molecular and phenotypic analysis. All experiments were performed following the guidelines of standard infection control procedures and biosafety level 2 (BSL-2) practices.

2-2 Serogrouping

Isolates of *E. coli* were serotyped by the slide agglutination test using an O-typing antiserum kit. Fresh bacterial colonies were grown on a normal saline suspension, and agglutination with polyvalent (P) and monovalent (M) antisera was performed to determine strains of the various O serogroups. Agglutination was visible in 1–2 min, and the suspension was removed with saline washings to avoid autoagglutination. Isolates without a reaction were classified as untypeable. Seroclassification results were analyzed in relation to the virulence genes and clinical data.

2-3 DNA Extraction:

Genomic DNA from *E. coli* isolates was extracted using a commercial kit with silica membranes (QIAamp® DNA Mini Kit, Qiagen, Hilden, Germany) following the manufacturer's instructions for Gram-negative bacteria. As a summary, one colony from an overnight blood agar culture was resuspended in 180 µL of enzymatic lysis buffer (Lysozyme 20 mg/mL) and incubated at 37°C for 30 min to disrupt the bacterial cell wall. Digestion with the proteinase K/AL buffer and flow-through samples, DNA was washed onto the spin-column membrane using ethanol precipitation. After two washes of AW1 and AW2 buffers to remove proteins, salts, and cell debris (Qiagen), pure genomic DNA was ultimately recovered in 100 µL preheated (70°C) AE buffer. DNA quantification and quality were assessed in a spectrophotometer (NanoDrop™ One, Thermo Fisher Scientific) with OD260/OD280 1.8-2.0 being considered acceptable. The extracted DNA was stored at –20 °C until used for PCR reactions

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2-4 Amplification of Virulence Genes by PCR:

The presence of virulence factor (VF) genes such as adhesins (*fimH, papC, sfa/focDE, dra/draBC)*, iron acquisition factors (*iutA, fyuA*), host serum resistance factors (*iss, traT*), and toxins (*cnf1, hlyA, sat vat*) was studied using multiplex PCR with earlier reported primers. The amplification was done from gene-specific oligonucleotide primers as listed in Table 1 for various major virulence determinants of extraintestinal pathogenic *E. coli* as described (Salman, 2025, p. 1-13; Hyun et al., 2021, p. 77; Huja et al., 2015; Al-Sa'ady et al., 2020, p. 100911). The PCR condition was set as follows: 95°C for 5 min, with denaturing at 95°C, annealing at 55–60°C (optimised according to the target gene), and extension at 72°C for each cycle with a final extension step at 72°C for another 5 minutes.

Table (1): Primers used for PCR amplification of virulence genes in septicemia-associated *Escherichia coli*

Virulence category	Gene	Primer (5′→3′)	Amplicon size (bp)	Reference
Adhesins	*fimH*	F: TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	508	(Salman, 2025, p. 1-13.)
	papC	F: GTGGCAGTATGAGTAATGACCGTTA R: ATATCCTTTCTGCAGGGATGCAATA	200	(Salman, 2025, p. 1-13.)
	sfa/focD	F:	410	(Salman

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	E	CTCCGGAGAACTGGGTGCATCTTAC R: CGGAGGAGTAATTACAAACCTGGCA		, 2025, p. 1-13.)
	afa/draBC	F: GGCAGAGGGCCGGCAACAGGC R: CCCGTAACGCGCCAGCATCTC	559	(Hyun et al., 2021, p. 77)
Iron acquisition	*iutA*	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300	(Hyun et al., 2021, p. 77)
	fyuA	F: TGATTAACCCCGCGACGGGAA R: CGCAGTAGGCACGATGTTGTA	880	(Hyun et al., 2021, p. 77)
Serum resistance	*iss*	F: AATTCCCGAAACGAAGAA R: ATGCTTATTACAAGGATG	323	(Huja, Sagi, et al., 2015, p. 10-1128)
	traT	F: GGTGTGGTGCGATGAGCACAG R: CACGGTTCAGCCATCCCTGAG	290	(Hyun et al., 2021, p. 77)
	cnf1	F:	498	(Hyun et

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Toxins		AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTATT		al., 2021, p. 77)
	hlyA	F: AACAAGGATAAGCACTGTTCTGGCT R: ACCATATAAGCGGTCATTCCCGTCA	1177	(Hyun et al., 2021, p. 77)
	sat	F: TCAGAAGCTCAGCGAATCATTG R: CCATTATCACCAGTAAAACGCACC	930	(Al-Sa'ady, et al., 2020, p. 100911 .)
	vat	F: AACGGTTGGTGGCAACAATCC R: AGCCCTGTAGAATGGCGAGTA	420	(Al-Sa'ady, et al., 2020, p. 100911 .)

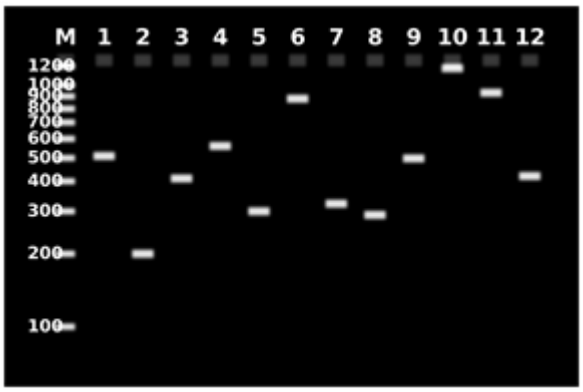

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Figure (1): Schematic representation of agarose gel electrophoresis (1.5% agarose) showing multiplex PCR amplification of virulence genes. Lane M: 100 bp DNA ladder (1200–100 bp). Lane 1: *fimH* (508 bp); Lane 2: *papC* (200 bp); Lane 3: *sfa/focDE* (410 bp); Lane 4: *afa/draBC* (559 bp); Lane 5: *iutA* (300 bp); Lane 6: *fyuA* (880 bp); Lane 7: *iss* (323 bp); Lane 8: *traT* (290 bp); Lane 9: *cnf1* (498 bp); Lane 10: *hlyA* (1177 bp); Lane 11: *sat* (930 bp); Lane 12: *vat* (420 bp).

2-4 Antibiotic Susceptibility:

Antimicrobial susceptibility testing was performed by the broth microdilution method (BMD) as per Clinical and Laboratory Standards Institute (CLSI, 2025) and European Committee on Antimicrobial Susceptibility Testing (EUCAST, v15.0, 2025) guidelines. MICs of 12 antimicrobial agents representing eight classes were tested.

E. coli isolates were cultured on Mueller-Hinton agar for 24 h at 37°C; an equivalent of a 0.5 McFarland suspension was prepared and then further diluted to yield ~5 × 10^5 CFU/mL in CA-MHB. After incubation at 37°C for 18–20 h, MIC values were recorded as the lowest concentration with complete inhibition of visible growth. The reference strains *E. coli* ATCC® 25922 and Pseudomonas aeruginosa ATCC® 27853 were included in all series of experiments as a quality control.

Isolates were defined as MDR (non-susceptible to ≥1 agent in ≥3 antimicrobial categories) according to the definition (Abdeta et al., 2021, p. e0256556). All values were analysed using the latest CLSI M100 (2025) and EUCAST breakpoint tables.

2-5 Statistical Analysis:

Chi-square assessed the association between bacterial features of serogroups, VFs, host factors of different clinical parameters, and outcome for sepsis severity and mortality, with a p-value < 0.05 as significant. In

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addition, this association was still remarkable after multivariate analysis (adjusted for potential confounding factors , including age, comorbidities, or length of stay at the ICU); SPSS v.26 was used to analyze data. Genotypes of high virulence, possessing ≥4 VFs, such as toxins and serum resistance genes.

3- Results:

3.1- Clinical and Demographic Characteristics in Relation to Pathogen Virulence:

Of the 86 adult patients with *E. coli* septicaemia (age: 20–70 yrs), mild sepsis was present in 48 (55.8%) and severe sepsis or septic shock in 38 (44.2%), of whom the inpatient mortality rate was 37.2% (n = 32). As shown in Table 2, some host factors, including age 60 to 70 years and older, DM, CKD, malignancy, ICU admission or mechanical ventilator use, and previous treatment with antibiotics, were significantly associated with severe illness ($p < 0.05$). However, these clinical features are not included as individual patient characteristics but as major confounders in our multivariate analysis of bacterial traits in relation to the adverse outcomes. It is noteworthy that the distribution of these host risk factors was non-random within pathogen subgroups. For instance, a larger number of VF genes and genotypes were significantly required for ICU (73.7% vs 12.5%, $p < 0.001$) and mechanical ventilation (60.5% vs 8.3%, $p < 0.001$), suggesting an interaction between microbial pathogenicity and host susceptibility in this disease process. A higher proportion of isolates from non-survivors were, however, phenotypically resistant to serum (84.4% versus 59.3%, $p = 0.018$) and predominantly carried O-serogroup (65.6% versus 40.7%, $p = 0.028$), highlighting the inadequacy of using severity scores without simultaneous pathogen profiling. Thereby, the clinical-demographic setting is not just presented as an end in itself but provides the necessary epidemiologic background to describe unique predictive values of *E. coli* virulence factors - a key focus of the current study.

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Table (2): Patient and pathogen characteristics according to sepsis severity and clinical outcome

Variable	All patients (n = 86)	Mild illness (n = 48)	Severe illness (n = 38)	*p*-value	Survivors (n = 54)	Non-survivors (n = 32)	*p*-value
20–34 yr.	14 (16.3)	11 (22.9)	3 (7.9)		12 (22.2)	2 (6.3)	
35–49 yr.	16 (18.6)	12 (25.0)	4 (10.5)		11 (20.4)	5 (15.6)	
50–59 yr.	26 (30.2)	14 (29.2)	12 (31.6)		15 (27.8)	11 (34.4)	
60–70 yr.	30 (34.9)	11 (22.9)	19 (50.0)	<0.001	16 (29.6)	14 (43.8)	0.018
Male	29 (33.7)	14 (29.2)	15 (39.5)	0.298	15 (27.8)	14 (43.8)	0.118
Female	57 (66.3)	34 (70.8)	23 (60.5)		39 (72.2)	18 (56.2)	
Diabetes mellitus	32 (37.2)	13 (27.1)	19 (50.0)	0.028	15 (27.8)	17 (53.1)	0.017
Hypertension	29 (33.7)	14 (29.2)	15 (39.	0.314	16 (29.6)	13 (40.6)	0.281

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Variable	All patients (n = 86)	Mild illness (n = 48)	Severe illness (n = 38)	*p*-value	Survivors (n = 54)	Non-survivors (n = 32)	*p*-value
			5)				
Chronic kidney disease	21 (24.4)	6 (12.5)	15 (39.5)	0.004	8 (14.8)	13 (40.6)	0.006
Chronic liver disease	12 (14.0)	4 (8.3)	8 (21.1)	0.087	5 (9.3)	7 (21.9)	0.083
Cardiovascular disease	26 (30.2)	10 (20.8)	16 (42.1)	0.031	13 (24.1)	13 (40.6)	0.094
Malignancy (any type)	17 (19.8)	4 (8.3)	13 (34.2)	0.002	6 (11.1)	11 (34.4)	0.006
Prior surgery (≤30 days)	22 (25.6)	7 (14.6)	15 (39.5)	0.009	9 (16.7)	13 (40.6)	0.011
ICU admission	34 (39.5)	6 (12.5)	28 (73.7)	<0.001	11 (20.4)	23 (71.9)	<0.001
Mechanical ventilation	27 (31.4)	4 (8.3)	23 (60.5)	<0.001	8 (14.8)	19 (59.4)	<0.001

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Variable	All patients (n = 86)	Mild illness (n = 48)	Severe illness (n = 38)	*p*-value	Survivors (n = 54)	Non-survivors (n = 32)	*p*-value
Immunosuppressive therapy	19 (22.1)	5 (10.4)	14 (36.8)	0.004	7 (13.0)	12 (37.5)	0.006
Prior antibiotic use (≤90 days)	41 (47.7)	17 (35.4)	24 (63.2)	0.010	20 (37.0)	21 (65.6)	0.009
High-virulence genotype	42 (48.8)	14 (29.2)	28 (73.7)	<0.001	18 (33.3)	24 (75.0)	<0.001

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Variable	All patients (n = 86)	Mild illness (n = 48)	Severe illness (n = 38)	*p*-value	Survivors (n = 54)	Non-survivors (n = 32)	*p*-value
≥1 toxin gene present	36 (41.9)	12 (25.0)	24 (63.2)	0.001	16 (29.6)	20 (62.5)	0.003
Serum resistance gene present	59 (68.6)	25 (52.1)	34 (89.5)	<0.001	32 (59.3)	27 (84.4)	0.018

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Variable	All patients (n = 86)	Mild illness (n = 48)	Severe illness (n = 38)	*p*-value	Survivors (n = 54)	Non-survivors (n = 32)	*p*-value
The iron acquisition gene is present	67 (77.9)	34 (70.8)	33 (86.8)	0.082	39 (72.2)	28 (87.5)	0.102
Dominant O-serogroups (O25/O6/O15)	43 (50.0)	16 (33.3)	27 (71.1)	<0.001	22 (40.7)	21 (65.6)	0.028

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Variable	All patients (n = 86)	Mild illness (n = 48)	Severe illness (n = 38)	*p*-value	Survivors (n = 54)	Non-survivors (n = 32)	*p*-value
Multidrug-resistant (MDR) phenotype	31 (36.0)	9 (18.8)	22 (57.9)	<0.001	12 (22.2)	19 (59.4)	<0.001

- Immunosuppressive therapy: corticosteroids, chemotherapy, or biologic agents
- High-virulence genotype: isolates carrying ≥4 virulence genes
- Toxin genes: *hlyA* and/or *cnf1*
- Serum resistance gene: *iss*
- MDR phenotype: resistance to ≥3 antimicrobial classes

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3.2- Virulence Gene Prevalence:

Multiplex-PCR clustering of the 86 *E. coli* isolates found a high occurrence of those with GA of virulence genes adhesin (detected in 80 isolates, 93%), iron acquisition system(s) (77.9%), serum resistance gene (68.6%), and toxin genes *hlyA* and *cnf1*. The majority of the isolates harboured virulence genes that were included in at least 3 functional categories, and this suggested a high pathogenic potential. A high-virulence genotype (≥4 virulence genes, one or more toxins (*hlyA or cnf1*), and the serum resistance gene (*iss*, *traT*)) was identified from 42 (48.8%) of isolates. Patients infected with a high-virulence strain had an approximately threefold risk of severe sepsis/septic shock (73.7% versus 29.2%; $p < 0.001$) and in-hospital death (75.0% versus 33.3%; $p < 0.001$). These associations were statistically significant after adjustment for age, comorbidities, ICU admission, and pre-admission antibiotic treatment, indicating that the bacterial virulence genotype is an independent predictor for poor outcome in *E. coli* bloodstream infection.

3.3- O-Serogroup Virulence:

All the 86 *E. coli* strains were serogrouped and divided into 11 O-types, with O25 (20.9%), O6 (16.3%), and O15 (12.8%) as the most prevalent serogroups, together accounting for 50% of total isolates, which will be designated as "dominant serogroups". The rest of the strains belonged to rare serogroups (O1, O2, O4, O7, O8, O16, O18, and O75) or were non-typeable (3.5%). In particular, strains of dominant serogroups O25, O6 , and O15 exhibited significantly higher carriage rates for major virulence factors: adhesins (>90%), iron acquisition systems (78–83%), serum resistance genes (71–78%), or toxin genes (*hlyA/cnf1*, 43–45%) (Table 3). However, untypeable or rare serogroup strains lacked enterotoxin genes and had significantly lower serum resistance ratios as well as iron acquisition systems. Clinically, the dominant serogroups were grossly overexpressed in severe sepsis patients (71.1% vs 33.3%, $p < 0.001$) and non-survivors (65.6% vs 40.7%, $p = 0.028$), which suggested that there was a strong trend

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between these O-serogroups, possession of virulence genes, and outcome status with coli septicaemia; they occur in a dose-dependent manner.

Table (3): Distribution of O-Serogroups and Virulence Factor (VF) Genes among *E. coli* Isolates Recovered from Sepsis Patients (n = 86)

O-Serogroup	No. of isolates n (%)	Adhesins n (%)	Iron acquisition n (%)	Serum resistance n (%)	Toxins n (%)
O25	18 (20.9)	17 (94.4)	15 (83.3)	14 (77.8)	8 (44.4)
O6	14 (16.3)	13 (92.9)	11 (78.6)	10 (71.4)	6 (42.9)
O15	11 (12.8)	10 (90.9)	9 (81.8)	8 (72.7)	5 (45.5)
O75	9 (10.5)	9 (100)	7 (77.8)	6 (66.7)	4 (44.4)
O8	7 (8.1)	6 (85.7)	5 (71.4)	4 (57.1)	3 (42.9)
O1	6 (7.0)	6 (100)	5 (83.3)	4 (66.7)	3 (50.0)
O18	5 (5.8)	5 (100)	4 (80.0)	4 (80.0)	2 (40.0)
O16	4 (4.7)	4 (100)	3 (75.0)	3 (75.0)	2 (50.0)
O2	3 (3.5)	3 (100)	2 (66.7)	2 (66.7)	1 (33.3)
O4	3 (3.5)	2 (66.7)	2 (66.7)	1 (33.3)	1 (33.3)
O7	3 (3.5)	3 (100)	2 (66.7)	2 (66.7)	1 (33.3)
Untypeable/ rare serogroups	3 (3.5)	2 (66.7)	2 (66.7)	1 (33.3)	0 (0)
Total	86 (100)	80 (93.0)	67 (77.9)	59 (68.6)	36

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O-Serogroup	No. of isolates n (%)	Adhesins n (%)	Iron acquisition n (%)	Serum resistance n (%)	Toxins n (%)
					(41.9)

3.4- Mortality Risk Factors:

Multivariate logistic regression analysis was conducted to establish in-hospital mortality predictors after adjusting for potential confounders such as age (>60 years), comorbidities (diabetes mellitus, chronic kidney disease, malignancy), ICU admission, mechanical ventilation use, history of antibiotics, and immunosuppressive therapy. The most predictive independent death factor was high-virulence genotype (v ≥ 4 factors, at least one toxin factor gene (hlyA or cnf1), and one resistance serum gene (iss or traT) (Odds Ratio OR: 5.21; CI95%: 2.04-13.31; P < .001). Furthermore, membership of the predominant O-serogroups (O25/O6/O15) was still associated with death after adjustment (adjusted Odds Ratio aOR = 3.18; 95% CI: 1.22–8.29; p = 0.018). Serum resistance determinants were also correlated with death (adjusted OR = 2.94; 95% CI: P. 028). MDR was not an independent prognostic factor in multivariate analysis, contrary to what might be expected as affecting poor outcome (p = 0.112), since it could be virulence and not resistance itself that would influence these series. These results emphasize the importance of combining pathogen genotype information with clinical history in risk stratification of patients with *E. coli* septicaemia.

3.5- Antimicrobial Resistance Patterns:

MDR (non-susceptible to ≥3 antibiotic classes) was observed among 31/86 (36.0%) *E. coli* BSIs. As shown in the heat map (Figure 2), MDR strains were frequently co-resistant to third- or fourth-generation cephalosporins, like ceftriaxone and cefepime, fluoroquinolones (ciprofloxacin, levofloxacin), and β-lactam/β-lactamase inhibitor combinations (piperacillin-tazobactam). Carbapenem (meropenem,

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ertapenem, or imipenem-cilastatin) resistance was observed in four to nine isolates (13–29% MDR; 5–10%), whereas ceftazidime-avibactam demonstrated activity with only two to three non-susceptible isolates (≤3.5%). By contrast, non-MDR isolates (n = 55) were highly susceptible to all drugs, indicating that wide drug resistance is restricted only to the MDR subset of this population.

Figure (2): Antibiotic Resistance and MDR Status Across 86 *E. coli* Bloodstream Isolates from Baghdad, Iraq (January 2025–January 2026).

4- Discussion:

Association of virulence factors and O-serogroups with severity of infection, antimicrobial resistance profiles, and outcome among sepsis caused by *E. coli* bloodstream infections (BSI) was the aim of this study in Baghdad, Iraq. We integrated bacterial genotypic profiling with detailed clinical data to determine whether pathogen-specific characteristics contribute independently to disease severity and in-hospital mortality beyond traditional host risk factors.

In the current study, 12 virulence genes (*fimH, papC, sfa/focDE, afa/draBC, iutA and fyuA; iss* and *traT; cnf1* and *hlyA; sat and vat*) were analysed with respect to their possible involvement in septicaemia isolates of *E. coli*, such that high composite virulence scores were noted for ESBL-producing blood isolates. Adhesins (*fimH, papC, sfa/focDE, and afa/draBC*) were well-represented, suggesting their indispensable role in adherence, and were

disseminated throughout the host. In addition, the iron acquisition receptors (*iutA* and *fyuA*) were detected in the majority of isolates, again providing evidence for the fitness advantage that ExPEC possess in low-iron serum biology. Genes of serum resistance (*iss* and *traT*) and toxin genes (*hlyA, cnf1, sat, vat*) were significantly associated with severe sepsis and mortality when they coexisted in at least four gene combinations, suggesting that the pathogenicity of multiple-gene virulence factors might be synergistically enhanced rather than a sole one.

These findings are consistent with other international studies that have reported high rates of *fimH*, *papC*, and iron acquisition systems among invasive ExPEC strains. Hyun et al. (2021, p. 77) and Kim et al. (2022, p. 203–212) also confirmed an enrichment of adhesin and iron acquisition genes in blood isolates compared to the non-invasive isolates. Moreover, D'Onofrio et al. (2023, p. 1827) found hlyA and cnf1 to be independently associated with increased severity of sepsis, consistent with our observation of toxin-positive isolates being over-represented among non-survivors. A second report from Iran (Hemati et al., 2024, p. 1426510) and our findings and those from Saudi Arabia (Bazaid, A. S., et al., 2022, p. 1907) have shown that the iss and traT genes were both linked with complement resistance and are a bad clinical outcome, further validating this discovery of serum resistome candidates.

Importantly, our data highlight that the cumulative virulence, rather than antimicrobial resistance alone, better predicted poor outcomes. This aligns with Daneman, Nick, et al. (2023, p. 56), who reported that mortality in *E. coli* bacteremia is more closely linked to pathogenic potential than resistance phenotype. In comparison with regional Iraqi studies that focused mainly on resistance patterns, the present work provides integrated molecular evidence linking adhesins, iron acquisition systems, serum resistance genes, and toxins to clinical severity. Collectively, these results reinforce the concept that comprehensive virulence profiling of these twelve genes may serve as a valuable adjunct for risk stratification and targeted management of *E. coli* septicaemia, particularly in resource-limited healthcare settings.

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The genes associated with serum resistance (*iss*, *traT*) appear to play a special role. The presence of serum resistance genes was significantly higher in patients with severe disease (89.5% versus 52.1%, both $p < 0.001$) and death (84.4% versus 59.3%, $p = 0.018$); condensing results are presented in Table 2. These gene products help to prevent killing by complement and prolong bacteremia with extension into the systemic circulation. Additional analogous studies were identified by Daneman, Nick et al. 2023, p. 56) - (Zhao, Shunjin, et al. 2022, p. 713-721), who identified serum resistance genes that corresponded with mortality in *E. coli* bacteremia. The relatively high prevalence of these risk factors among severe patients, as observed in our population, indicates their importance from a biological perspective.

Toxic genes (*hlyA and cnf1*) were more frequently recovered from patients with severe disease than from other patients (63.2% versus 25.0%, $p = 0.001$). The vasoactive toxins are responsible for endothelial damage, inflammatory responses, and organ dysfunction. The autotransporter toxins, however (*Sat*, *Vat*), were not as frequent and had only poor predictive power when compared to the classical cytotoxins in bloodstream infections.

The importance of biological severity biomarkers has been emphasised in recent publications. Alshammary et al. (2025, p. 4) found the immune and biochemistry laboratory biomarkers that largely determined both the severity of sepsis and increased mortality, thus indicating an additive value to having objective biologic markers in prognostic models. In a similar vein, molecular profiling of virulence by *E. coli* isolates in this study may be used as an alternative surrogate prognostication indicator and is applicable to aid in predicting the prognosis, particularly in an environment where state-of-the-art biomarker testing is not readily available.

The resistance pattern revealed that 36%, as in Figure 2, were multidrug resistant (MDR), an alarming level of cephalosporin and fluoroquinolone resistance. However, MDR (OR 2.33) was not a significant independent predictor of mortality ($p = 0.112$), indicating that virulence had a stronger association with outcome than sterilisation therapy. Alshammary et al.

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(2025, p. 4) observed that in vitro markers of disease severity will frequently precede microbiological indicators, and indeed, it was suggested that cumulative virulence could result in clinical deterioration, particularly where AMR is high, as well as in settings (resources) (ICIUM). According to Table 2, the conventional risk factors for severe sepsis (age ≥60, diabetes, chronic kidney disease and cancer) and death (age >60, cancer, ICU admission requiring mechanical ventilation and previously receiving an antibiotic) appeared to be statistically significant with adverse outcomes (all $p < 0.05$), which are in line with some reports on sepsis at the international level (Chen et al. 2026; Ziaian et al. 2025). One interesting finding was that virulence type independently predicted outcomes; high-virulence types (≥4 virulence genes, RFLs) were more frequently found in severe cases and non-survivors and retained a statistically significant association with death (aOR = 5.21; 95% CI: 2.04–13.31; $p < 0.001$).

Serogrouping also emphasises the importance of pathogen genotype on disease outcome. The most dominant serogroups (O25, O6, and O15) made up 50% of isolates with a high prevalence of carriage of adhesins (>90%), iron acquisition systems (78 to 83%), and serum resistance genes (71 to 78%), but also toxin genes (42–45%; Table 3). Clinically, these serogroups were strikingly more prevalent in severe cases (71.1% vs 33.3%, $p < 0.001$) and among non-survivors (65.6% vs 40.7%, $p = 0.028$), as detailed in Table 2. This straightforward correlation between Tables 2 and 3 shows that some of the O-serogroups are enriched for virulence determinants associated with a bad outcome in patients.

These findings are in line with national and global publications. Kolenda, Rafał, et al. (2021, p. 000743) found that O25 was a prevalent serogroup that caused severity of systemic infection in Germany, and Bazaid, A. S., et al. (2022, p. 1907) found that multidrug-resistant UPEC strains harbouring more virulence genes were significantly correlated with ICU admission and mortality in Saudi Arabia. Our results have confirmed and expanded these previous observations within the Iraqi setting; O25, O6, and O15 contributed to form together a major hypervirulent cluster with sepsis severity implications.

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Several limitations should be acknowledged. No whole-genome analysis was performed, and thus clonal assignments were not possible (e.g., identification of ST131 on O25 isolates). It is also limited to a relatively small sample size (n = 86), which restricts detailed subgroup analysis. However, the fusion of molecular and clinical data provides valuable information on the pathogen-host interaction of *E. coli* sepsis in resource-limited settings.

5- Conclusion:

Indeed, in cases of *E. coli* septicaemia, high virulence (≥4 VF genes, including toxin and serum resistance factors) and dominant O-serogroups (O25, O6, or O15) are a strong independent predictor for severe disease, as, for instance, they are not significantly different from MDR status or traditional host risk factors. These patients are also older adult patients with devastating infections from Baghdad, Iraq. These data reinforce the concept that molecular pathogen profiling can add value to clinical risk stratification and suggest possible utility for intervention-guiding virulence-based testing in resource-poor environments to support early antimicrobial stewardship. Inclusion of virulence scoring in routine clinical care for sepsis has the potential to improve prognosis and , hence, ultimately outcomes in geographies that are unable to offer high-level critical care for all.

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