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Original article

Study of genetic characterisation of multidrug-nonsusceptible *Escherichia coli* from septicaemia: Pulsed-field gel electrophoresis (PFGE) and PCR techniques

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ABSTRACT

Background: *Escherichia coli* (*E. Coli*) can cause septicemia, which is a deadly infection. Furthermore, the options for eradication have been restricted by the presence of virulence factors and multidrug resistance. This study aimed to detect phylogenetic groups, assess clinically important virulence genes, and determine the genetic relatedness of isolates**. Methods:** A total of 100 isolates of multidrug-resistant *E. Coli* were taken from septicemia. The disk diffusion method was used to test for antibiotic susceptibility. Polymerase chain reaction (PCR) was used to screen for virulence genes, carbapenemases, and extended-spectrum β-lactamases. Additionally, serogrouping and phylogenetic grouping were examined. Pulsed field gel electrophoresis was used for the genetic classification (PFGE). **Results:** Tetracycline, ceftazidime, and cefepime resistance was present in all isolates. There were 56, 55, and 43% of patients resistant to imipenem, meropenem, and gentamicin, respectively. The *blaCTX-M1* (51%), *blaSHV* (64%), *blaIMP* (33%) and *blaOXA*-48-like (12%) genes were present in the isolates. In 22%, 17%, 36% and 25% of cases, the phylogroups A, B1, B2, and D were found respectively. O1 accounted for 53% of the total serogroups, with O25 at 14% and O75 at 11%. The virulence genes *csgA, iutA, HlyA, traT, and KpsMII* were present in all of the isolates. Of the strains carrying *blaCTX-M1*, 80% ($p<0.0001$) had the O1 serogroup and 62% ($p<0.001$) belonged to the B2 phylogroup. Five clusters (A–E) with an 80% cut-off and no genetic relationship were shown by the PFGE. **Conclusion:** It is imperative to implement surveillance and appropriate control measures to stop the spread of drug-resistant and virulent *E. Coli* from septicemia.

Introduction

E. coli is able to colonize and persist in a wide range of environmental habitats as well as animal hosts. In addition to symbiotic relationship with their host, *E. coli* and other intestinal flora bacteria also help to create important signals that are necessary for the immune system's development and regulation as well as the host's defense against

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infections. [1]. Certain *E. coli* strains spread from their natural habitat and seriously infect hosts. These variants are categorized as diarrhegenic *E. Coli* or extra-intestinal *E. coli* (ExPEC); causing UTIs, prostate inflammation, bacteremia, septicemia, and neonatal meningitis [2, 3].

E. Coli-induced septicemia affects people of all ages. Those who have had surgery, an appendix that has become inflamed, or an intestinal tract perforation after an accident are most likely to have septicemia [4]. Patients with UTIs may also experience kidney infections, which can lead to septicemia [5]. Older ISEcp1adults, diabetics, immunocompromised people (such as transplant recipients, cancer chemotherapy or corticosteroid recipients), and patients with acquired immunodeficiency syndrome are among the patients with severe or fatal sepsis [6]. Severe sepsis, a systemic inflammatory reaction to infection, has a 20–40% fatality rate. The mortality rate is decreased by sepsis management and early diagnosis. Although it accounts for only 5% of UTI cases, severe sepsis is also frequently linked to pulmonary and abdominal infections; however, it accounts for nearly 40% of hospital infections [7].

Consequently, sepsis patients need to be identified quickly in order to stop them from developing into other organs or organ disorders. UTI is the most often used precursor for sepsis, and strains of the disease show resistance to several antibiotics [8, 9].

Gram-negative bacteria's primary defense mechanism against beta-lactam antibiotics is beta-lactamase production. After being first discovered in Japan in 1986, *CTX-M* beta-lactamase subsequently spread to other countries. [10]. *E. coli* strains can be categorized into four distinct phylogenetic groups, labeled A, B1, B2, and D, using PCR-based methods [11]. Phylogenetic analysis, a widely employed technique in molecular epidemiological studies of pathogenic *E. coli*, has revealed this classification based on genetic variations among the strains [12].

Insertion sequences (IS), which are add-on sequences, are one of the many factors that can prevent the transfer of *blaCTX-M* genes. These IS can insert into multiple locations within the target molecule and have a simple genetic organization, usually measuring between 0.8 and 1.8 kb (less than 2.5 kB). These sequences exhibit genetic compression despite their small size. They typically encode only the functions necessary for their mobility, such as the identification and processing of these endpoints by a transposal enzyme and the reactive DNA sequences that terminate the element. [13, 14].

The production of ESBLs enzymes enzymes are classified into different classes based on their origin and genetic similarity, most notably the TEM, SHV, CTX-M, OXA, and VIM and IPM enzymes. CTX-M enzyme as the most common type of ESBLs, particularly in Europe and North America. The gene encoding this enzyme is located adjucent to the mobile elements known as *ISEcp1* upon conjugative plasmids (25–150 kb) carried by isolates from various sources [15]. *ISEcp1, IS26 and ISCR1* appear to play a leading role in the acquisition of *CTX-M* genes in connection with class 1 Integron structures [16]. Furthermore, is a promoter region leading to the upregulation of the *CTX-M* gene.

The aim of this study is to detection of the phylogenetic groups among isolates, investigate the occurrence of genes encoding clinically important virulence genes, and determination the genetic relatedness of isolates by using pulsed field gel electrophoresis (PFGE) technique

Materials and methods

1- Bacterial Isolates selection and sampling sites

Sample size determination and sampling technique:

A single proportion formula was used to calculate sample size,

n=z2 p(1-p)/d2; p=3 %, z=1.96 , d=0.05, Z2 = abscissa of the normal curve that cuts off an area (α) at the tails (1.96)

d = the acceptable sample error at 95% confidence interval (0.05),in this study used 0.05 ,

p = the estimated proportion of the attribute present in the population (3%)

N=100.

A retrospective cross sectional study was conducted in which one hundred non-duplicated MDR-*E. coli* isolates, were collected from patients with septicaemia across various wards in AL-sadder hospital -Najaf governorate, from May to October 2023.The identification of *E. coli* isolates was performed using common biochemical reactions, and the isolates were then preserved in trypticase soy broth (TSB) with 30% glycerol at -20° C for subsequent analysis.

2- Antibiotic susceptibility test

The antibiotic susceptibility testing was conducted using the disk diffusion method (Kirby-Bauer) in accordance with CLSI recommendations. A panel of 13 antibiotics, including fosfomycin (FO 200µg), ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg), co-amoxiclav (AMC 30µg), cefazolin (CZ 30µg), nitrofurantoin (NI 300µg), piperacillin-tazobactam (PTZ 110µg), gentamycin (GM 10µg), tetracycline (TE 30µg), cotrimoxazole (TS 25µg), imipenem (IMI 10µg), meropenem (MEN 10µg), and ciprofloxacin (CIP 5µg), were employed. For precision, *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25922 standard strains were culture and used as controls during the test.

3- Phylogenetic Typing

Phylogenetic grouping based on *chuA* and *yjaA* genes, along with the DNA fragment TspE4.C2, was conducted for each strain through PCR [17]. The specific primers and PCR conditions are detailed in Table 1. The DNA extraction step was conducted following the manufacturer's guidelines (Favorgen, Taiwan), which involved isolating DNA from bacterial cells for subsequent analysis or experiments. The primers sequence and PCR condition were as mentioned by Dallenne et al., 2010 [18].

4- Detection of *bla*-genes

Escherichia coli isolates were screened for the presence of *bla*-genes, namely *bla*TEM, *bla*SHV, *bla*CTX-1, *bla*IMP, *bla*OXA-48, and *bla*VIM, by monoplex PCR [19]. The primer sequences and PCR conditions were adopted from previous references [18, 20, 21, 22], as described in **Table 2**. Amplicons were separated by agarose gel electrophoresis using 1.5% (w/v) agarose gel and stained with ethidium bromide. Positive results were detected when the DNA band size of the sample matched the target product size. The PCR reactions were prepared in a total volume of 25 µl, consisting of 10 µl of PCR master mix (Solg™mix), 2 µl of each primer, and 5 µl of the extracted DNA, with the volume adjusted to 25 µl using sterile deionized distilled water. The biometra PCR system (T3000 Thermocycler) was utilized to detect the presence of the target genes.

5- O-serogroup typing

The most prevalent serogroups of ExPEC, including O1, O2, O4, O6, O7, O12, O15, O16, O18, O25, O75, and O157, were identified using PCR to target the corresponding antigen-encoding genes. Details regarding the specific primers and temperature conditions can be found in Table 3. A uni-plex PCR reaction was used in which the protocol involved an initial denaturation step of the extracted DNA lasting 5 minutes at 94°C, followed by 30 cycles of 1 minute at 94°C, 1 minute at 61°C, and 50 seconds at 72°C. The reaction concluded with a final extension step lasting 10 minutes at 72°C.

6- **Virulence genes**

This study explored a variety of virulence genes, including Toll/interleukin 1 receptor (*TcpC*),), capsular polysaccharide synthesis K1 (*kpsMTII*), ferric aerobactin receptor (*iutA*), cytolethal distending toxin (cdt cytotoxic necrotizing factor 1 (*cnf1*),), vaculating autoinducer toxin (*vat*) α-hemolysin (*hlyA*), conjugal transfer gene (*traT*), invasion of brain endothelium (*ibeA*), curli fimbriae (csgA, secretion autoinducer toxin (*sat*) and serine protease autoinducer (*pic*). Details regarding the specific primers and temperature conditions can be found in Table 4.

7- Pulsed field gel electrophoresis (PFGE) according to manufacturer instructions

8- To create a microbial suspension, isolates kept at -70°C were first cultivated on TSA medium in the PFGE process. After that, the isolates were cultured overnight in MHA medium, and the next steps involved making DNA and plugs. Low-melting agarose was combined with a microbial suspension to create plugs. The plugs were transferred into test tubes, and a lysis buffer composed of proteinase K, sarcosine, and EDTA was added. This mixture was incubated in a shaking incubator at 55°C for three hours. Subsequently, each plug underwent a cleaning process involving two washing steps with a TE-buffer solution, followed by three rinses with distilled water.

An endonuclease was used in two stages of the enzymatic digestion process. The plugs were first incubated for an hour at 37°C after being combined with distilled water and enzyme buffer. They were then combined with an endonuclease mixture, enzyme buffer, and distilled water. This was followed by an overnight incubation at 37°C. After that, 1% agarose gel electrophoresis was carried out in 1X TBE buffer. The gel was next stained with ethidium bromide, cleaned in distilled water in a gel Doc, and then it was analyzed. The DNA fingerprint band patterns generated by Pulsed

field gel electrophoresis (PFGE) were analyzed using GelJ software (reference). GelJ is a convenient tool for constructing phylogenetic trees and dendrograms to analyze gel electrophoresis DNA fingerprints. clustering analysis was performed with GelJ software based on the dice similarity coefficient and the unweighted pair group method with arithmetic mean (UPGMA) to assess the discriminatory power of **PFGE**.

9- Statistical analysis

Statistical analysis and comparison of the results were conducted using SPSS software version 20. Excel 2014 statistical software was also employed for further data analysis. Qualitative data underwent analysis using $\chi 2$ (Chi-square test) and Fisher-Mann-Whitney U-test. Variables with a P value <0.05 were considered significant in the analysis.

Results

1- Bacterial isolates

One hundred MDR-ExPEC isolates (resistant to to tetracycline, ciprofloxacin, ceftazidime and co-trimoxazole) were detected from septicemic patients. Common biochemical tests were used to figure out the isolates. Clinical samples were collected from hematology (65%), intensive care unit (ICU) and cardiac care unit (CCU) (5%), renal Unit, urology and nephrology (14%), gastroenterology (9%), and cardiology, respiratory, pulmonary diseases, rheumatology, and bone marrow transplant unit wards (7%). The septicemic patients included 45 males (mean age of 54.44) and 55 females (mean age of 47.21) with age ranging from 1-90 years old. Prior antibiotic usage was observed among 79 (p<0.001) of them.

2- Antibiotic suscetibilty results :

The relation of antibiotic resistance and existence of beta-lactamase genes has been shown in the table 5. All the isolated strains were nonsusceptible to tetracycline, ceftazidime and cefepime. The imipenem, meropenem and gentamicin resistance included 56, 55 and 43%, respectively.

3- Phylogroups distribution

Among the 100 isolates, 36% were assigned to the B2 phylogroup, 25% for D, 22% for A, and 17% for the B1 phylogroup. As shown in the **figure 1**, every isolate that had the *bla*VIM gene belonged to the A phylogroup. Moreover, the pattern of *CTXM-1, TEM* and *OXA-48* were in the B1 phylogroup and the pattern *CTXM-1, TEM, SHV* and *IMP* was in the B2 phylogroup. The isolates carried the $bla_{\text{CTX-M1}}$ (51%), bla_{SHV} (64%), bla_{IMP} (33%) and $bla_{\text{OXA-48-like}}$ (12%) genes. Of $bla_{\text{CTX-M1}}$-bearing strains, 62% (p<0.001) belonged to the B2 phylogroup.

4- Serogroup typing in *E. coli* isolates

The O1 (53%), O25 (14%) and O75 (11%) serogroups were most frequently encountered.The distribution of serogroups among phylogroups was in disparity without significant association (p>0.05, table 6 Q5). Of $bla_{\text{CTX-M1}}$-bearing strains, 80% (p<0.0001) had O1 serogroup.

5- virulence typing

Among 100 isolates from septicemia, all carried the *iutA*, *HlyA*, *TraT*, and *KpsMII* virulence genes. However, *csgA* was present in 50% of the isolates. Of the 51 CTX-M-bearing isolates, eighteen belonged to phylogroup B2. The association of virulence genes and resistance profiles is depicted in Table 7. Accordingly, the bla_{VIM} gene was among all the isolates carrying the *iutA, csgA, ibeA, vat, traT* and *hlyA* genes. Moreover, CTX-M1+TEM profile was significantly associated with the *csgA* (88.8%, p<0.001), *traT* (88.8%, p<0.001) and *hlyA* (88.8%, p<0.001) genes. CTX-M1+SHV profile was significantly associated with *iutA* (71.4%, p<0.001), *csgA* (85.7%, p<0.001), *traT* (71.4%, p<0.001) and *hlyA* (85.7%, p<0.001). Notably, CTX-M1+TEM +OXA-48 pattern had a similar association, but the CTX-M1+TEM+SHV+IPM had also further associations with *kpsMII* and *tcpC* genes. In addition, CTX-M1+TEM +SHV+OXA-48 profile had significant association with the *iutA, kpsMII, csgA, traT, sat, hlyA* and *pic* genes (100% for each, p<0.0001).

6- Pulsed field gel electrophoresis (PFGE)

In the current research, the PFGE depicted five clusters (A-E) using 80% cut-off without any genetic relation. Hence, surveillance and proper control strategies are essential to prevent the distribution of virulent and drug-resistant *E. coli* from septicemia. There was a high genetic variation without relation among *E. coli*. The PFGE pattern of CTX-M producing isolates has been exhibited in **figure2**.

In the clustering, the results of PFGE, five clusters (A-E) were created considering a 80% cut off. A cluster contained 12 isolates, all of which having the *ISEcp1* upstream of the *CTX-M* gene. Additionally, 83% of them (A cluster) belonged to

the hematological ward, 66% of them were from males and 58% of them contained serogroup A.

Cluster B consisted of 10 isolates, half of which were from males, 80% of them had CTX-M gene.

The cluster C consisted of 11 isolates, most of which belonged to the B2 phylogroup, and two isolates belonging to the D group that had of their *CTX-M* gene.

The cluster D included 9 isolates, 8/9 having gene. The interesting point about this cluster was that there were two isolated gastroenterology isolates, both belonging to the D phylogroup

Finally, the cluster E is the same as the cluster D, which has 9 isolates. In this cluster, two isolates belonging to the A phylogroup, both of which are related to the hematological ward, are located on the of the *CTX-M* β-lactamase gene, which has *ISEcp1*. Two isolates in the upstream of *CTX-M* gene have of which are male and belong to the B2 phylogroup.

Table1. The primers used for phylogenetic grouping

Primer	Sequence
ChuA	F-5′-GACGAACCAACGGTCAGGAT-3′ R-5′-TGCCGCCAGTACCAAAGACA-3′),
YjaA	(F-5′-TGAAGGTCAGGAGACGCTG-3′, R-5′-TGGAGAATGCGTTCTCAAC-3′),
TspE4C2	(F-5′-GAGTAATGTCGGGGCATTCA-3′, R-5′-CGCGCCAACAAAGTATTACG-3′).

Table2. The primers and PCR conditions used for the detection of resistance genes in this study

Target	Primer name	Primer sequence (5´-3´)	Product (bp)	Reference
***bla*TEM**	TEM -F	ATGAGTATTCAACATTTCCG	867	Rasheed *et al*., 1997
	TEM- R	CTGACAGTTACCAATGCTTA		
***bla*SHV**	SHV-F	GGGTTATTCTTATTTGTCGC	930	
	SHV-R	TTAGCGTTGCCAGTGGTC		
***bla*CTX-M1**	CTX-M-1-F	AGT TCA CGC TGA TGG CGA CG	676	Bogaerts *et al.*,2007
	CTX-M-1-R	AAC CCA GGA AGC AGG CAG TCC		
***bla*VIM**	VIM-F	GATGGTGTTTGGTCGCATA	390	Dallenne et al., 2010
	VIM-R	CGAATGCGCAGCACCAG		
*bla*IMP	IMP-F	TTGACACTCCATTTACDG	139	
	IMP-R	GATYGAGAATTAAGCCACYCT		
***bla*OXA-48**	OXA-48-F	TTG GTG GCA TCG ATT ATC GG	744	Poirel *et al.*, 2004
	OXA-48-R	GAG CAC TTC TTT TGT GAT GGC		

Table 3. Primers used for detection of O-serogroups

Primers	Sequences 5′-3′	PCR product size (bp)
gndbis.F	5′-ATACCGACGACGCCGATCTG-3′	-
rfbO1.R	5′-CCAGAAATACACTTGGAGAC-3′	189
rfbO2.R	5′-GTGACTATTTCGTTACAAGC-3′	274
rfbO4.R	5′-AGGGGCCATTTGACCCACTC-3′	193
rfbO6.R	5′-AAATGAGCGCCCACCATTAC-3′	584
rfbO7.R	5′-CGAAGATCATCCACGATCCG-3′	722
rfbO12.R	5′-GTGTCAAATGCCTGTCACCG-3′	239
rfbO15.R	5′-TGATAATGACCAACTCGACG-3′	536
rfbO16.R	5′-GGATCATTTATGCTGGTACG-3′	450
rfbO18.R	5′-GAAGATGGCTATAATGGTTG-3′	360
rfbO25.R	5′-GAGATCCAAAAACAGTTTGTG-3′	313
rfbO75.R	5′-GTAATAATGCTTGCGAAACC-3′	419
rfbO157.R	5′-TACGACAGAGAGTGTCTGAG-3′	672

Table 4. Primers used for detection of virulence genes

Primers	Sequences 5′-3′	PCR product size (bp)
cdt	FP: 5′-AAATCACCAAGAATCATCCAGTTA-3′ RP: 5′-AAATCTCCTGCAATCATCCAGTTTA-3′	430
kpsMTII	FP: 5′-GCGCATTTGCTGATACTGTTG-3′ RP: 5′-CATCCAGACGATAAGCATGAGCA-3′	272
TcpC	FP: 5′-GAGTGGAAGGAGGTTGAGGC-3′ RP: 5′-GCAGTGCCATTTTATCCGCC-3′	544
iutA	FP: 5′-GGCTGGACATCATGGGAACTGG-3′ RP: 5′-CGTCGGGAACGGGTAGAATCG-3′	302
traT	FP: 5′-GGTGTGGTGCGATGAGCACAG-3′ RP: 5′-CACGGTTCAGCCATCCCTGAG-3′	290
hlyA	FP: 5′- GCATCATCAAGCGTACGTTCC-3′ RP: 5′- AATGAGCCAAGCTGGTTAAGCT-3′	534
cnf1	FP: 5′- AAGATGGAGTTTCCTATGCAGGAG-3′ RP: 5′- CATTCAGAGTCCTGCCCTCATTAT-3′	498
ibeA	FP: 5′- AGGCAGGTGTGCGCCGCGTAC-3′ RP: 5′-TGGTGCTCCGGCAAACCATGC-3′	171
vat	FP: 5′- AACGGTTGGTGGCAACAATCC-3′ RP: 5′- AGCCCTGTAGAATGGCGAGTA-3′	420
sat	FP: 5′- TCAGAAGCTCAGCGAATCATTG-3′ RP: 5′- CCATTATCACCAGTAAAACGCACC-3′	330
pic	FP: 5′- ACTGGATCTTAAGGCTCAGGAT-3′ RP: 5′- GACTTAATGTCACTGTTCAGCG-3′	572
CsgA	FP: 5′- GGCGGAAATGGTTCAGATGTTG-3′ RP: 5′- CGTATTCATAAGCTTCTCCCGA-3′	295

Table 5. The relation of antibiotic resistance and beta-lactamase genes (%):

Gene/Antibiogram	TS	IMI	MEN	GM	FO	PTZ	AMC
VIM	100	100	100	100	50	50	100
CTX-M1+TEM	77.7	0	0	44.4	0	11.1	77.7
CTX-M1+SHV	57.1	0	0	85.7	14.2	0	85.7
CTX-M1+TEM +SHV	64.5	0	0	61.2	3.2	3.2	74.1
CTX-M1+TEM +OXA-48	100	0	0	100	0	0	100
CTX-M1+TEM +SHV+IMP	0	100	100	0	0	0	0
CTX-M1+TEM +SHV+OXA-48	50	0	0	50	0	0	100

fosfomycin (FO), co-amoxiclav (AMC), piperacillin-tazobactam (PTZ), gentamicin (GM), imipenem (IMI), meropenem (MEN)

Table 6. The association of major O-serogroups and phylogroups

O serogroups	Phylogroup A	Phylogroup B1	Phylogroup B2	Phylogroup D
O1 (n=53)	3/53	12/53	17/53	21/53
O25 (n=14)	4/14	8/14	2/14	0.0
O75 (n=11)	9/11	2/11	0.0	0.0
O7 (n=9)	4/9	3/9	1/9	1/9
O2 (n=4)	1/4	0.0	1/4	2/4

Table 7. The association of virulence genes and existence of resistance genes

Resistance/virulence	*cnf*	*cdt*	*iutA*	*csgA*	*Kps MII*	*ibe A*	*vat*	*Tra T*	*tcp C*	*sat*	*hlyA*	*pic*
VIM	0	50	100	100	50	100	100	100	0	0	100	0
CTX-M1+TEM	11.1	0	66.6	88.8	55.9	11.1	0	88.8	0	11.1	88.8	22.2
CTX-M1+SHV	28.5	0	71.4	85.7	57.1	0	0	71.4	0	42.8	85.7	14.2
CTX-M1+TEM +SHV	16.1	3.2	64.5	90.3	45.1	0	3.2	67.7	0	38.7	83.8	12.9
CTX-M1+TEM +OXA-48	0	100	100	0	0	0	0	100	0	0	100	0
CTX-M1+TEM+SHV+IPM	0	0	100	100	100	0	0	100	0	100	100	100
CTX-M1+TEM +SHV+OXA-48	0	0	100	50	50	0	0	100	0	0	100	0

Figure1. The association of phylogroups and resistance genes

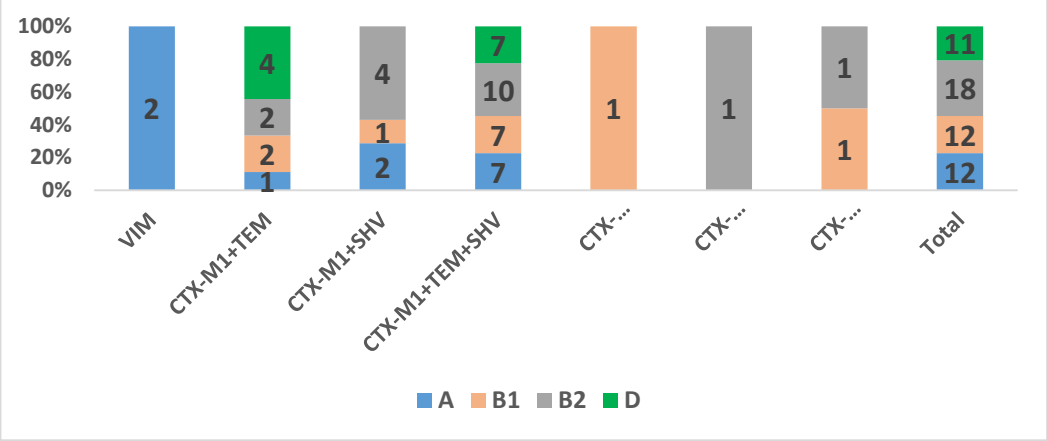
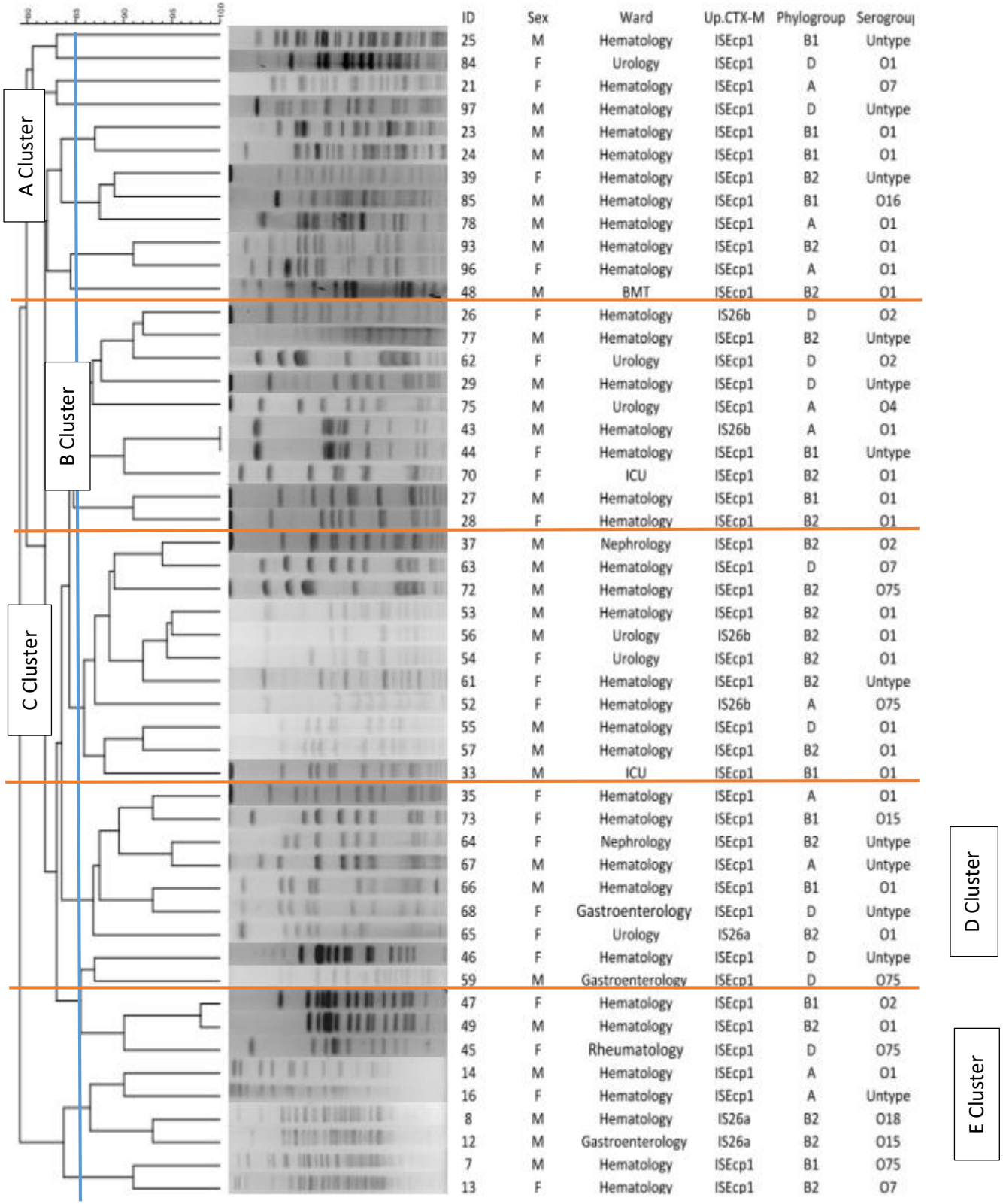


Figure 2. The PFGE dendrogram, generated through XbaI digestion for CTX-M positive isolates, was constructed based on the dice similarity coefficient and the UPGMA pattern using gel-compar software. This dendrogram specifically represents the 51 CTX-M producing isolates. A blue line serves as the 80% cut-off point, revealing a diverse distribution of isolates. The analysis identified 50 distinct patterns. (M, male, F, female, up *CTX-M*, upstream of *CTX-M* genetic-environmental (*ISEcp1*, *IS26a*, and *IS26b*).



Discussion

Particularly, urinary tract infections, septicemia, visceral ulcers, liver abscesses, cholecystitis, pancreatic abscesses, and lower respiratory tract infections are frequently caused by *E. coli.* Fatal infections are caused by isolates that contain carbapenemases and ESBLs. In this study, all the isolates were resistant to tetracycline, ceftazidime and cefepime. The imipenem, meropenem and gentamicin resistance included 56, 55 and 43%, respectively. Additionally, none of them were resistant to fosfomycin. Inadequate monitoring and control of antibiotic use may lead to resistance and the failure of future infection treatments. Concerns regarding the potential spread of antibiotic resistance are further raised by the ciprofloxacin and gentamicin resistance found in this study. Totally, resistance to used antibiotics was relatively high. The spread of ESBL-bearing *E. coli* has been demonstrated among previous studies [23, 24]. The patterns of antibiotic consumption, geographic location, variations in the patterns of resistance in various regions, and the unchecked use of antibiotics in every country are all factors that influence the variations in antibiotic resistance patterns.

We observed that the isolates carried the $bla_{\text{CTX-M1}}$ (51%), bla_{SHV} (64%), bla_{IMP} (33%) and $bla_{\text{OXA-48-like}}$ (12%) genes. Among CTX-M-bearing *E. coli*, the *ISEcp1* was detected upstream of the CTXM1 gene in 43/51 (84.3%) of them. Moreover, the *IS26b* and *IS26a* were detected in 5 (9.8%) and 3 (5.8%) isolates, respectively. Additionally, the *orf477* was detected downstream of five isolates.

Systemic infections caused by *Escherichia coli* involve multiple virulence factors. This is noteworthy because there is a crisis in the eradication of infections due to the co-transfer of virulence factors and ESBLs by a considerable number of ESBL-bearing *E. coli* from different sources. [19]. Noticeably, in the present study, the *hlyA* gene (86%) was predominant, followed by *csgA*, *traT*, *iutA* and *kpsMII*, being 84%, 79%, 70% and 57%, respectively. In actuality, five virulence genes were present in more than 50% of the strains. Human cells are directly harmed by necrotizing cytotoxic factor and alpha hemolysin (HlyA), while *sat*, *pic*, and *vat* genes increase the risk of septicemia infection. Additionally, adhesin genes trigger *E. Coli* strains' colonization and biofilm formation. [25, 26].

Of 100 isolates, 36% of them belonged to the B2 phylogroup, followed by 25% D, 22% A, and 17% to the B1 phylogroup. all the isolates carrying the bla_{VIM} gene belonged to the A phylogroup. Moreover, the pattern of CTXM-1, TEM and OXA-48 were in the B1 phylogroup and the pattern CTXM-1, TEM, SHV and IPM was in the B2 phylogroup. Of $bla_{\text{CTX-M1}}$-bearing strains, 62% ($p<0.001$) belonged to the B2 phylogroup. Studies have exhibited that most of the extra-intestinal pathogenic strains belong to phylogroup B2 and D [27].

In addition, the O1 (n=53), O25 (n=14) and O75 (n=11) serogroups were predominant, followed by O7 (n= 9 %), O2 (n= 4%). The distribution of serogroups among phylogroups was in disparity without significant association. Of $bla_{\text{CTX-M1}}$-bearing strains, 80% ($p<0.0001$) had O1 serogroup. Recently O25 and ESBL-producing virulent clonal groups have been isolated which lead to therapy failure [28, 29].

In the genetic clustering, the results of PFGE with a cut off 80% of the five clusters A-E were created. A cluster containing 12 isolates, all of which were upstream of the *CTX-M* gene, having the *ISEcp1* insertion element. Serogroup A was present in 58% of the samples, 66% of the isolates were male, and 83% of the isolates were from the hematological ward. We found no genetic relationship between the isolates in the PFGE that were causing septicemia, indicating that there was no comparable source of outbreak or spread. Several studies had similar results, but some have demonstrated outbreak of pathogenic ESBL-bearing *E. coli* from healthcare and community [16, 30 - 32]. The control programs must be implemented to hinder the spread of these strains. It is necessary to put the control programs into place to prevent these strains from spreading. We observed the effectiveness of fosfomycin in treating ESBL-producing *E. Coli* infections.

Conflict of interest:

There is no conflict of interest.

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Conclusion

High levels of antibiotic resistance were found in this study's ESBL-producing *E. Coli* from sepsis, underscoring the necessity of surveillance and effective control measures to stop their spread.

The importance of these strains, which have been isolated from sepsis, as a critical infection is further highlighted by the co-existence of ESBL enzymes and virulence factors (primarily *hlyA*, csgA, and *traT* genes), as observed in this study. Furthermore, the B2 and D phylogroups accounted for the majority of isolates. The lack of genetic relationships between the ESBL-producing sepsis-related *E. coli* isolates indicates that there is no shared source of infection between them.

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