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Original Research Article

Allelic Resolution of *β Lactamase* Genes and Clonal Heterogeneity in Multidrug Resistant *E. coli* from Clinical Specimens in Iraq

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Abstract: ***Background*:** Resistance to β lactamase by Escherichia coli is one of the principal molecular mechanisms leading to failure of extended spectrum cephalosporin antibiotics. Nevertheless, family-based PCR results are often considered evidence of ESBL phenotype despite the non-ESBL parent gene identified by sequencing. ***Objectives*:** Analysis of clinical isolates collected from Iraq using a molecular genetic approach considering alleles, including antimicrobial susceptibility testing, multiplex PCR, partial gene sequencing, phylogenetic analysis and ERIC PCR fingerprinting. ***Methods:*** Fifty-two isolates were characterized by conventional identification, disk diffusion, phenotypic ESBL and AmpC tests, multiplex PCR targeting *bla* TEM, *bla* SHV, *bla* CTX-M, *bla* OXA, and six plasmid-mediated AmpC families, partial Sanger sequencing, and ERIC-PCR. Data were analyzed using Wilson 95% confidence intervals, Cohen's κ, and exact McNemar tests. ***Results*:** The highest resistance frequencies were observed for tetracycline (67.3%), trimethoprim/sulfamethoxazole (55.8%), and nalidixic acid (42.3%); 40.4% of isolates met the definition of multidrug resistance (MDR; resistance to ≥3 antimicrobial classes). Thirty-five isolates (67.3%) carried at least one screened *β-lactamase*-family marker. *bla*TEM was detected in 29 isolates (55.8%), *bla*OXA in eight (15.4%), *bla*SHV in one (1.9%), *bla*DHA in three (5.8%), and *bla*CTX-M in none. Sequencing assigned the detected alleles to TEM-1, SHV-1, OXA-1, and DHA-1. Consequently, the molecular results demonstrate *β-lactamase* gene carriage but do not justify labeling all PCR-positive isolates as ESBL producers. Phenotype–marker concordance was poor for the ESBL comparison (42.3%; $\kappa = -0.03$; $p = 0.0014$) and limited for AmpC (71.2%; $\kappa = 0.025$; $p = 0.0074$). ERIC-PCR resolved 16 profiles among 35 gene-positive isolates at 50% similarity, indicating substantial clonal heterogeneity. ***Conclusion*:** Allele discrimination essentially alters the way family level PCR results should be analyzed. In order to properly classify resistance and perform surveillance in an Iraqi medical setting, both current phenotypic criteria and allele discrimination sequencing must be used.

Keywords: *Escherichia Coli*, *B-Lactamase*, AmpC, *bla*TEM-1, *Bla*OXA-1, *Bla*DHA-1, ERIC-PCR.

1. INTRODUCTION

Antimicrobial Resistance (AMR) poses a significant risk to public health around the world. If there are no effective methods to prevent further spread, the number of deaths caused by AMR microorganisms is estimated to increase drastically over the next decades. Recent studies performed within the framework of the Global Burden of Disease project estimate that bacterial AMR will continue to be one of the leading causes of death worldwide until 2050 [1]. To address the rising issue, the WHO has included third generation cephalosporin-resistant Enterobacterales in a list of Critical Priority Pathogens [2]. Nonetheless, surveillance data available for many low- and middle-income countries, including Iraq, are limited, which makes it hard to assess the epidemiology of resistance in Gram-negative bacteria.

Escherichia coli is one of the most important members of Gram-negative bacteria, as it naturally inhabits the human gut and acts as a causative agent of numerous infections ranging from UTIs to blood infections, as well as being an excellent reservoir for mobile genetic elements responsible for antibiotic resistance. Third generation cephalosporin resistance is often caused by the presence of extended-spectrum beta lactamases (ESBLs), AmpC plasmid mediated *β-lactamases*, increased expression of ampC chromosome, alterations in outer membrane permeability, or some combination

of these factors. Modern systems for classifying *β-lactamases* consider both biochemical and molecular features of these enzymes and not just their belonging to gene families [3, 4].

Global prevalence of ESBL producing *E. coli* is determined primarily by CTX-M enzymes, yet the distribution of resistant strains depends largely on successful bacteria clones and plasmids that allow these strains to spread between the healthcare settings and internationally. Genome-wide studies revealed that even identical phenotypes of antibiotic resistance may emerge due to various types of resistance genes, alleles, mutations in promoters, plasmids, or clones [5-9]. Similarly, just knowing what gene is responsible for resistance cannot predict its phenotype due to regulation and genetic context of the gene. Likewise, PCR tests focused only on the gene family cannot detect clinically relevant alleles [6, 7].

For the TEM, SHV, and OXA families in specific, this deviation is crucial. Although OXA 1 is a limited spectrum class D β lactamase and not a canonical ESBL on its own, TEM 1 and SHV 1 are classical broad-spectrum penicillinases rather than ESBL differences. Allele identification or functional evidence is required to determine ESBL activity in these families, which is dependent on particular amino acid changes [3, 4]. Therefore, there is a risk of systematic overclassification if a positive blaTEM, blaSHV, or blaOXA amplicon is understood as an ESBL genotype without sequencing. Another aspect associated with AmpC beta-lactamases is their identification. Genes such as blaDHA-1 or blaCMY-2 present in *E. coli* can result in phenotype that simultaneously generates ESBLs, porin loss, or ampC promoter mutations on the chromosome. This implies that modern diagnostic approaches include both specific tests and genome sequencing [5-12]. The present study was conducted to perform a molecular genetic characterization of clinical *E. coli* isolates recovered from patients in Iraqi hospitals. The objectives were to: (i) quantify the antimicrobial resistance profile; (ii) determine the frequency of screened β lactamase family markers; (iii) resolve sequenced amplicons to allele level; (iv) assess concordance between phenotypic and molecular classifications; and (v) characterize clonal diversity by ERIC PCR.

2. MATERIALS AND METHODS

Experimental Design and Isolate Collection

This is a retrospective study based on an anonymous laboratory database that consists of 52 unique strains of clinical *E. coli* isolated from patients suspected to have UTIs at tertiary hospitals in Iraq. Among these, 44 isolates (84.6%) were collected from urine samples while 8 isolates (15.4%) were collected from vaginal swabs. No information about any institution, city name, laboratory names or other geographical/occupational identifiers was provided in this study and there were no patient identifiers either. Ethics approval was obtained from the ethics committees of the participating hospitals, and patient data were de-identified prior to analysis.

Bacterial Identification

Isolates were cultured on MacConkey and eosin methylene blue media. Presumptive identification was confirmed by Gram staining and standard biochemical reactions, including indole production, methyl red, Voges-Proskauer, citrate utilization, motility, hydrogen sulfide production, and triple sugar iron reactions. The expected profile was a Gram-negative, motile, indole-positive, methyl-red-positive, Voges-Proskauer-negative, citrate-negative bacillus producing acid/acid reactions with gas and without hydrogen sulfide.

Antimicrobial Susceptibility Testing

Disk diffusion was performed on Mueller-Hinton agar with ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), kanamycin (30 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). A 0.5 McFarland suspension was used. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. Results are reported as categorical findings and must not be used for current clinical decisions because breakpoint tables and quality-control requirements evolve; contemporary laboratories should apply the current CLSI M100 standard [15].

Phenotypic Detection of ESBL and AmpC Activity

The ESBL screen used cefotaxime and ceftazidime alone and in combination with clavulanic acid. An increase of at least 5 mm in inhibition-zone diameter in the presence of clavulanate was interpreted as a positive ESBL phenotype. AmpC activity was screened using cefotaxime and ceftazidime with and without phenylboronic acid; an increase of at least 5 mm with boronic acid was interpreted as a positive AmpC phenotype.

DNA Extraction and Multiplex PCR

Genomic DNA was prepared from overnight cultures by a boiling-lysis procedure. Multiplex PCR screened for four *β-lactamase* families commonly associated with ESBL or related resistance (*bla*TEM, *bla*SHV, *bla*CTX-M, and *bla*OXA) and six plasmid-mediated AmpC groups (MOX/CMY-1-like, CIT/CMY-2-like, DHA, ACC, EBC/MIR-ACT, and FOX). Amplicons were resolved on 1.5% agarose gels. Primer sequences and expected product sizes are summarized in Table 1.

Table 1: Oligonucleotide primers used for multiplex PCR detection of *β-lactamase* families and ERIC-PCR fingerprinting

Target	Forward primer (5′→3′)	Reverse primer (5′→3′)	Expected product (bp)
MOX/CMY-1-like	GCT GCT CAA GGA GCA CAG GAT	CAC ATT GAC ATA GGT GTG GTG C	520
CIT/CMY-2-like	TGG CCA GAA CTG ACA GGC AAA	TTT CTC CTG AAC GTG GCT GGC	462
DHA	AAC TTT CAC AGG TGT GCT GGG T	CCG TAC GCA TAC TGG CTT TGC	405
ACC	AAC AGC CTC AGC AGC CGG TTA	TTC GCC GCA ATC ATC CCT AGC	346
EBC/MIR-ACT	TCG GTA AAG CCG ATG TTG CGG	CTT CCA CTG CGG CTG CCA GTT	302
FOX	AAC ATG GGG TAT CAG GGA GAT G	CAA AGC GCG TAA CCG GAT TGG	190
SHV	ATG CGT TAT ATT CGC CTG TG	TGC TTT GTT ATT CGG GCC AA	747
TEM	TCG CCG CAT ACA CTA TTC TCA GAA TGA	ACG CTC ACC GGC TCC AGA TTT AT	445
CTX-M	ATG TGC AGY ACC AGT AAR GTK ATG GC	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593
OXA	ATT ATC TAC AGC AGC GCC AGT G	TGC ATC CAC GTC TTT GGT G	296
ERIC	ATG TAA GCT CCT GGG GAT TCA C	AAG TAA GTG ACT GGG GTG AGC G	Variable

Amplicon Sequencing and Phylogenetic Analysis

Representative PCR products were purified and sequenced by Sanger dideoxy termination. Consensus sequences were compared with curated GenBank entries using sequence-similarity searches. Multiple-sequence alignment was performed with ClustalW, and a Neighbor-Joining tree was reconstructed from a 282-bp aligned region using the Tajima-Nei distance model and 1,000 bootstrap replicates using MEGA X. This tree was used solely to confirm allele identities and does not represent a strain-level phylogeny. The sequences were deposited in GenBank under accession numbers KT336739-KT336757.

ERIC-PCR Typing

Enterobacterial repetitive intergenic consensus PCR was applied to the 35 isolates carrying at least one screened *β-lactamase* marker. Band presence or absence was scored as binary data using the Dice similarity coefficient. Similarity was summarized by unweighted pair-group analysis with arithmetic mean (UPGMA), and clusters were defined at a 50% similarity threshold. ERIC-PCR was interpreted as a low-resolution fingerprinting method rather than a substitute for whole-genome phylogeny.

Statistical Analysis

Resistance and gene frequencies were expressed as counts, percentages, and Wilson 95% confidence intervals. Phenotype–marker agreement was evaluated from the 2×2 tables using observed agreement, Cohen's κ, and the exact McNemar test. Because isolate-level covariates were unavailable, no regression or gene-resistance association model was attempted. Multidrug resistance was defined as resistance to at least three antimicrobial classes according to Magiorakos *et al*., [23]. Statistical analyses were performed using SPSS version 22.0 and MedCalc version 19.0.

3. RESULTS

Isolate Composition and Antimicrobial Resistance

All 52 isolates exhibited the expected phenotypic and biochemical profile of *E. coli*. The resistance distribution was non-uniform (Table 2 and Figure 1). Tetracycline resistance was highest at 67.3% (35/52; 95% CI 53.8-78.5), followed by trimethoprim/sulfamethoxazole at 55.8% (29/52; 95% CI 42.3-68.4) and nalidixic acid at 42.3% (22/52; 95% CI 29.9-55.8). Resistance to cefotaxime and ciprofloxacin was 30.8% each. Ceftazidime resistance was lowest at 11.5% (6/52; 95% CI 5.4-23.0). The dataset classified 40.4% (21/52) of isolates as MDR (resistant to ≥3 antimicrobial classes).

Table 2: Antimicrobial susceptibility profile of 52 clinical *Escherichia coli* isolates

Antimicrobial	Resistant, n/N	Resistance (%)	95% CI (%)
Tetracycline	35/52	67.3	53.8-78.5
Trimethoprim/sulfamethoxazole	29/52	55.8	42.3-68.4
Nalidixic acid	22/52	42.3	29.9-55.8
Cefotaxime	16/52	30.8	19.9-44.3
Ciprofloxacin	16/52	30.8	19.9-44.3
Ceftriaxone	13/52	25.0	15.2-38.2
Kanamycin	13/52	25.0	15.2-38.2
Norfloxacin	9/52	17.3	9.4-29.7
Ceftazidime	6/52	11.5	5.4-23.0

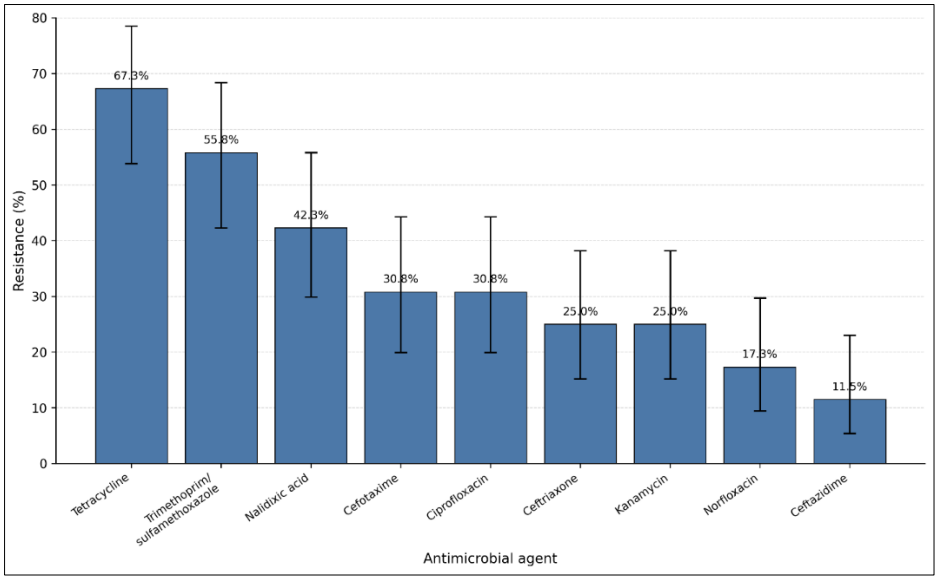

Figure 1: Bar chart illustrating the percentage of resistant isolates (n = 52) for each tested antimicrobial agent. Error bars represent 95% confidence intervals calculated using the Wilson method. Susceptibility was determined by disk diffusion following CLSI guidelines

***B-Lactamase*-Family Marker Carriage and Allele Assignment**

At least one screened *β-lactamase*-family marker was detected in 35/52 isolates (67.3%; 95% CI 53.8-78.5). The most frequent target was *bla*TEM (29/52; 55.8%), followed by *bla*OXA (8/52; 15.4%), *bla*DHA (3/52; 5.8%), and *bla*SHV (1/52; 1.9%). No *bla*CTX-M product was detected (Table 3 and Figure 2). Three isolates carried both *bla*TEM and *bla*OXA. All three *bla*DHA-positive isolates also carried at least one screened class A or D *β-lactamase* marker. Representative multiplex PCR products are shown in Figure 3. Panel A illustrates the amplicons corresponding to TEM, OXA, combined TEM/OXA, and SHV family targets, while panel B demonstrates the detection of the DHA family product in three isolates, with no amplification in the negative control (Figure 3).

Partial sequencing identified TEM-1, SHV-1, OXA-1, and DHA-1. This allele-level result is decisive: TEM-1 and SHV-1 are broad-spectrum parent enzymes, and OXA-1 is not a canonical ESBL. Accordingly, the 67.3% family-marker frequency must not be presented as a molecularly confirmed ESBL prevalence. The data support carriage of *β-lactamase* determinants, while the phenotypic clavulanate-synergy result remains the direct evidence of ESBL-like activity in this dataset.

Table 3: Prevalence of *β-lactamase* gene families and corresponding allele variants identified by partial sequencing among clinical *E. coli* isolates

Gene target	Positive isolates, n/N	Prevalence (%)	95% CI (%)	Allele identified
Any *β-lactamase* marker	35/52	67.3	53.8–78.5	Not applicable
*bla*TEM	29/52	55.8	42.3–68.4	TEM-1
*bla*OXA	8/52	15.4	8.0–27.5	OXA-1
*bla*DHA	3/52	5.8	2.0–15.6	DHA-1
*bla*SHV	1/52	1.9	0.3–10.1	SHV-1
*bla*CTX-M	0/52	0.0	0.0–6.9	Not detected

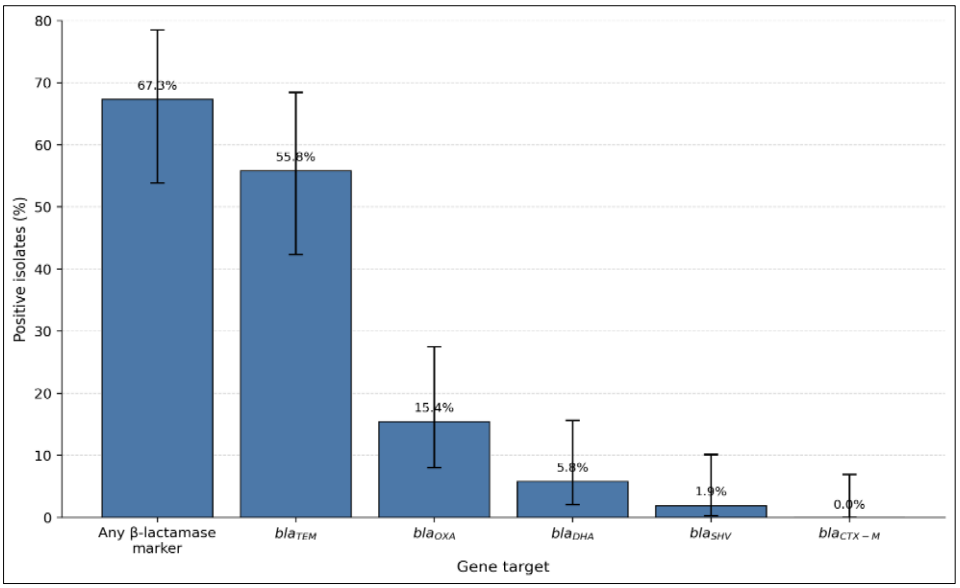

Figure 2: Prevalence of *β-lactamase* gene families detected by multiplex PCR among 52 clinical *E. coli* isolates, with Wilson 95% confidence intervals

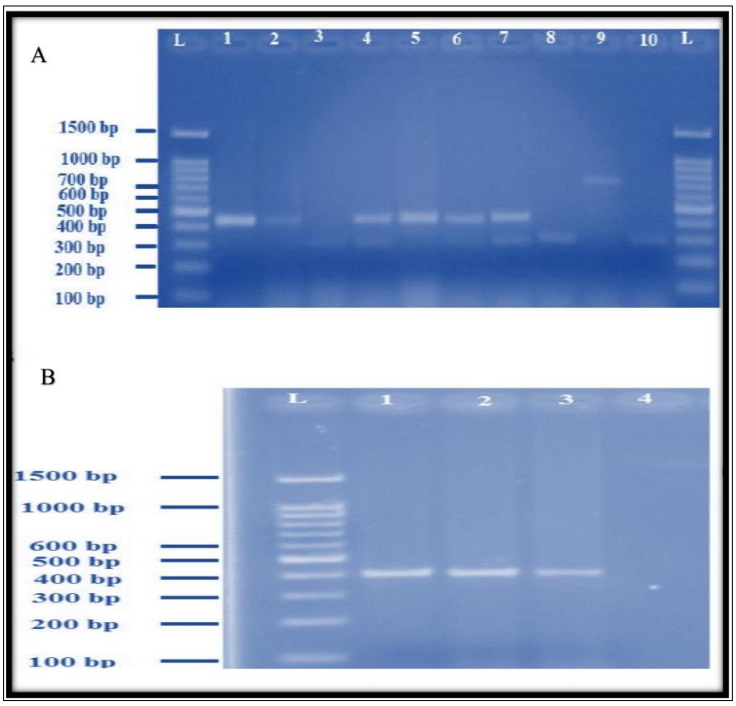

Figure 3: Representative multiplex PCR products for *β-lactamase* gene families in clinical *E. coli* isolates from Iraq. (A) Agarose gel electrophoresis showing PCR amplification of ESBL-related *β-lactamase* genes (blaTEM, blaSHV, blaOXA, and blaCTX-M). The lanes with "L" designation contain the DNA ladder. The selected amplified products consist of blaTEM (445 bp), blaOXA (296 bp), blaTEM + blaOXA, and blaSHV (747 bp). (B) Multiplex PCR detection of plasmid-encoded AmpC *β-lactamase* gene groups. Three clinical isolates produced the expected amplicon of DHA (405 bp) while the negative control sample (Lane 4) showed no amplification.

Discordance of Phenotype and Markers

The comparison of phenotypic ESBL test and molecular screening for ESBL-related genes with PCR showed high levels of discordance between the two methods. Using clavulanate synergy test, the ESBL phenotype was found in 17 isolates (32.7%), while the presence of ESBL marker by PCR was established in 35 isolates. There were 11 positive results common for both methods. On the other hand, 24 isolates had at least one molecular marker of ESBL production but lacked phenotypic confirmation. In addition, there were six isolates with positive ESBL phenotype but lacking all the screened ESBL markers. Eleven isolates had negative results for both ESBL phenotype and ESBL molecular markers. Overall agreement between the two methods was 42.3%. Cohen's κ was −0.03, indicating the absence of agreement and the exact

McNemar test confirmed significant difference between paired results (p = 0.0014). A similar trend was seen when comparing phenotypic detection of AmpC and screening for the blaDHA marker. Testing with phenotypic method identified 14 isolates (26.9%) as AmpC producers, while PCR identified just three isolates as possessing blaDHA marker. Only one isolate had a positive result by both tests; there were two isolates carrying blaDHA gene without phenotypic confirmation of AmpC production. Finally, 13 isolates had positive phenotypic test but lacking blaDHA marker. Thirty-six isolates had negative results for both methods. While overall agreement was 71.2%, Cohen's κ showed very low concordance (0.025). This is because of unequal distribution of negative and positive results and not because of agreement. This difference was proven by exact McNemar test (p = 0.0074).

Phylogenetic Classification of *β-Lactamase* Alleles

The phylogenetic analysis performed using the Neighbor-Joining approach revealed that the sequenced alleles belong to the respective reference variants of TEM-1, SHV-1, OXA-1, and DHA-1 (Figure 4). It is noteworthy that the TEM-1 branch is characterized by relatively high bootstrap value, whereas the OXA-1 and DHA-1 sequences are represented by independent branches. These data confirm the results of assignment of the alleles based on the sequence comparison performed within the 282-bp region. However, it must be kept in mind that the tree obtained does not represent the phylogeny of the bacteria strains since it was built using the partial sequences of the *β-lactamase* gene.

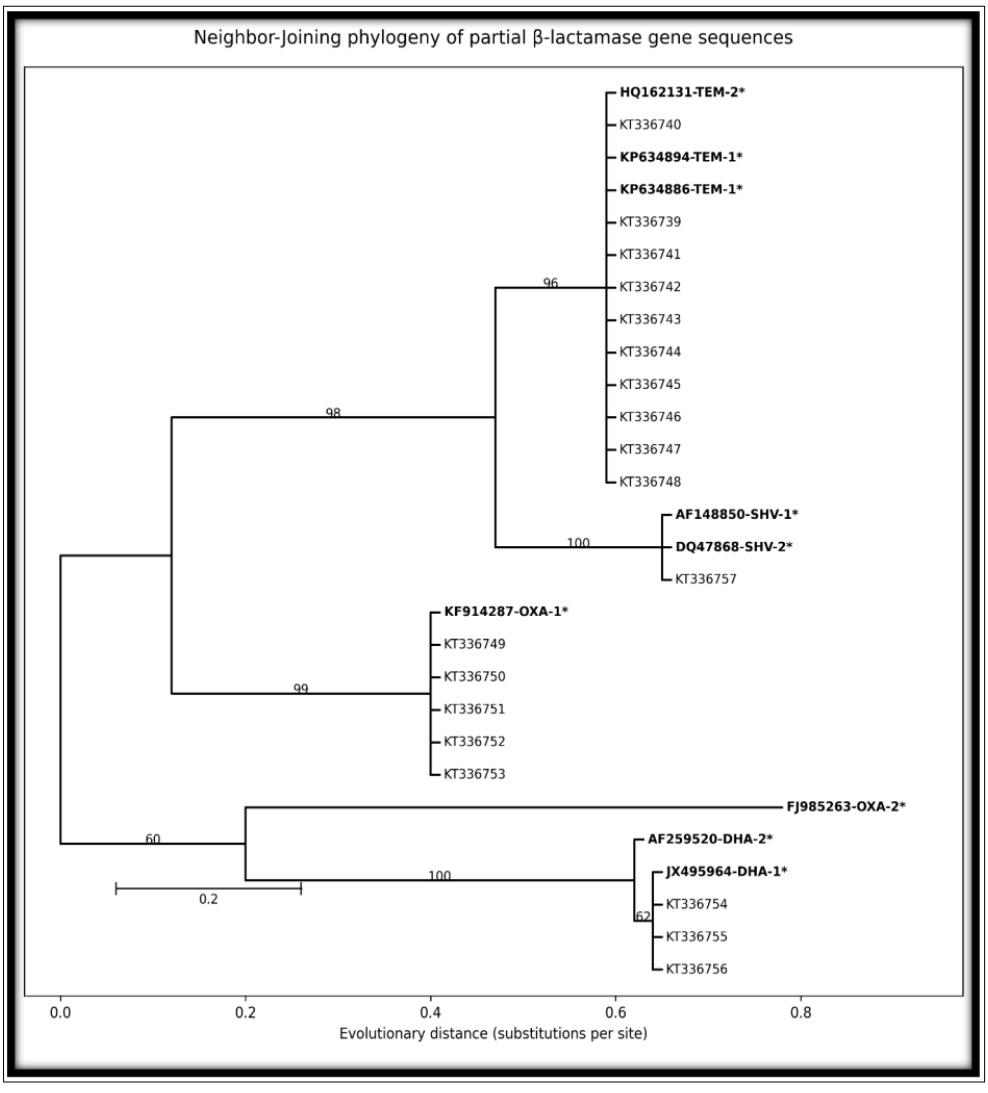

Figure 4: Neighbor joining phylogenetic tree developed from the partial *β-lactamase* gene sequences (282 bp) of clinical Escherichia coli isolates from Iraq using 1,000 replicate bootstrap support values

Clonal Diversity through ERIC-PCR

The ERIC-PCR analysis revealed the complex and non-homogeneous patterns among 35 isolates that contained markers (Figure 5). The dendrogram generated by the UPGMA method at the 50% similarity level (Dice coefficient) included 16 fingerprints (Figure 6). There were several small clusters, but no predominant one was identified. Thus, the results suggest genetic diversity of the isolates.

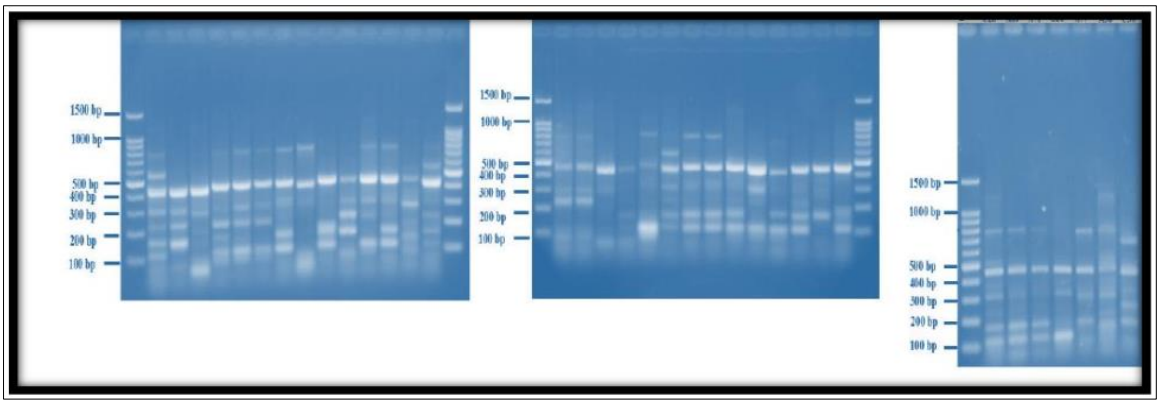

Figure 5: Represents ERIC-PCR fingerprint patterns from 35 *β-lactamase*-positive clinical Escherichia coli strains isolated in Iraq. The agarose gel electrophoresis shows ERIC-PCR amplification fingerprints of each strain. Lanes L represent the DNA molecular size markers, whereas all other lanes are individual isolates. The variations seen in the pattern of bands reveal great genomic variability in the isolates as seen in ERIC-PCR analysis. Identical fingerprints were not found, which means that the strains are genetically diverse

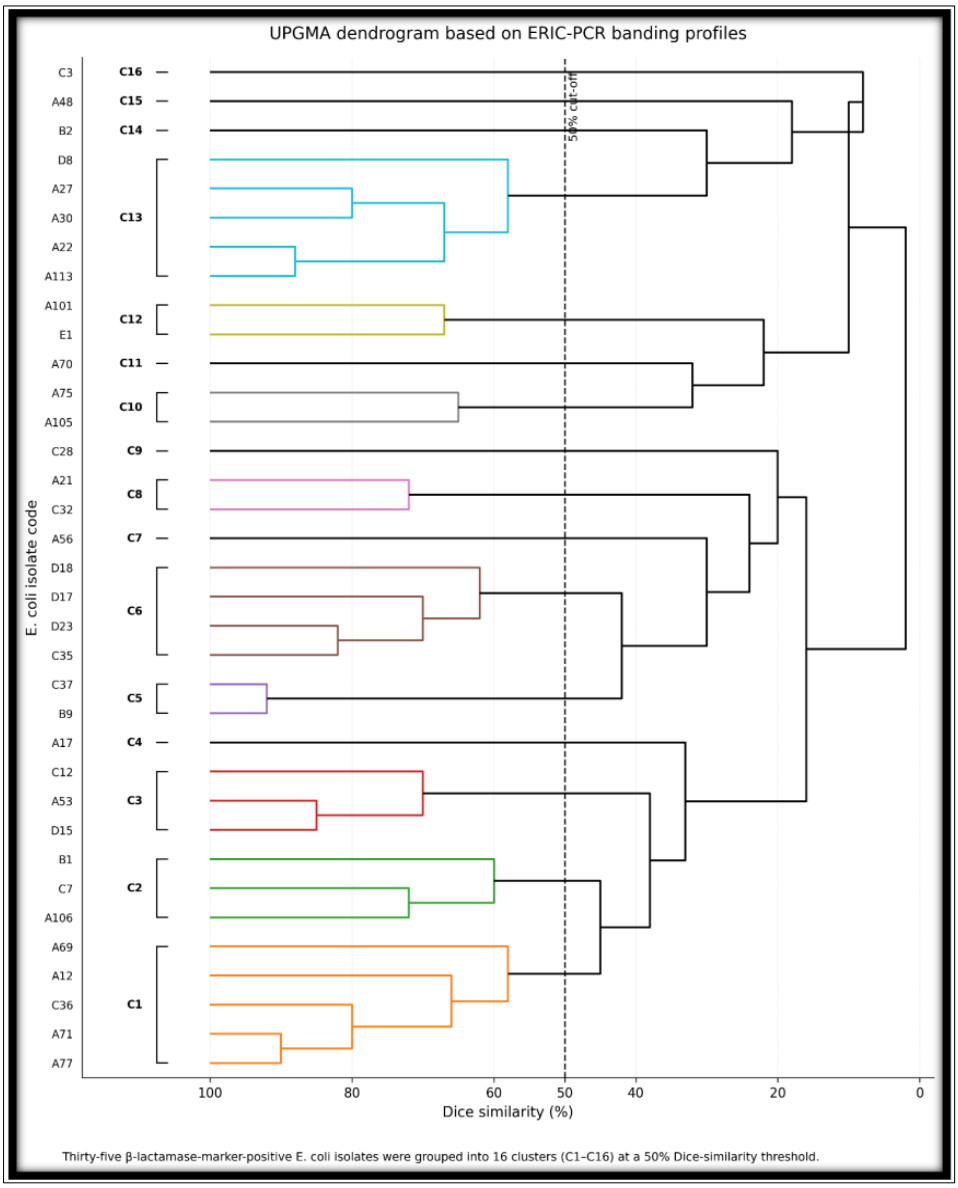

Figure 6: Dendrogram constructed by UPGMA showing the genetic relatedness between 35 clinical Escherichia coli strains positive for *β-lactamase* as obtained by ERIC-PCR fingerprinting

The dendrogram was made on the basis of UPGMA algorithm by ERIC-PCR patterns, where genetic similarity was calculated using the Dice coefficient. At a similarity value of 50%, 16 different clusters (C1-C16) could be recognized. Although many small clusters were identified, no single clone predominated. This is an indication of significant genetic diversity in the population. Identifiers of the isolates are given at the tips of the dendrogram, while geographical data have been excluded for confidentiality.

4. DISSCUSION

In this study, the molecular analysis provides a more detailed insight into β-lactam resistance among clinical isolates of Escherichia coli from Iraq compared to what would be obtained by family-based PCR detection alone. A high occurrence of *β-lactamases* was found along with resistance to multiple other classes of antibiotics not in the β-lactam class. The sequence analysis showed that TEM-1, OXA-1, SHV-1, and DHA-1 were the most common alleles. From the above observations, it is clear that family-based *β-lactamases* detection cannot be considered as proof of ESBLs production. On the contrary, the molecular data show that 67.3% of the isolates contained one of the detected *β-lactamase* family markers, while ESBLs were shown by the phenotypic clavulanate test.

This distinction is crucial in molecular genetics. The *β-lactamase* family includes different types of alleles. The primer set designed for amplification of a region in blaTEM gene will not distinguish TEM-1 from ESBLs if the amplified DNA fragment will not include the respective mutations and undergo sequencing analysis. Similar constraints are applicable to SHV and OXA families as well. Modern classification considers the enzymes whose functions have been defined on the level of sequence data [3, 4]. Therefore, sequencing identifies another interpretational mistake.

In spite of the wide geographical spread and variety of CTX-M enzymes discovered lately [4-8], no amplification of blaCTX-M was found in the current study. There are several reasons for such an outcome. It could be supposed that the isolates used for analysis comprise the population of bacteria where CTX-M enzymes are rare. Also, the nucleotide changes in the analyzed fragment could lead to decreased efficiency of primer binding, low quality of DNA could be responsible for the results, or there could be some resistance genes other than the ones tested in this study in the ESBL-positive isolates. At the same time, the fact that there were six isolates that showed positive phenotype for ESBL production but negative results for all tested molecular markers suggests that not all resistant alleles could be detected. Therefore, the lack of amplification using targeted PCR is not an indicator of the absence of blaCTX-M genes or other resistance mechanism.

The commonality of TEM 1 is quite reasonable biologically speaking, because TEM 1 continues to be one of the most common plasmid-associated penicillinases, and often accompanies genes for resistance to sulfonamides, trimethoprim, tetracyclines, or aminoglycosides. Therefore, the high levels of resistance to tetracycline and to trimethoprim/sulfamethoxazole can be due to either linkage through mobile elements or convergent evolution under similar selective pressures; nevertheless, the total data set does not provide any proof of genetic linkage. To determine whether these genes are on the same plasmid, transposon, or integron, complete genome sequencing is necessary [5-7].

Detection of DHA-1 in three isolates is of molecular importance irrespective of its low prevalence rate. DHA-1 is an inducible and plasmid-mediated class C beta-lactamase that may be responsible for the inactivation of extended-spectrum cephalosporins and can be found on mobile elements containing IS26 [11-14]. The 13 isolates that showed positive AmpC phenotype but negative plasmid markers could be due to mutations of chromosomal ampC promoter/attenuator, porin mutations, false-positive inhibitors, and introduction of different AmpC families that are difficult to amplify. Modern diagnostics also reveal that phenotypic tests of AmpC are unable to provide the explanation of genetics involved without molecular testing [11, 12].

These inconsistencies between ESBL phenotype and marker positivity could be explained by two reasons. First, the presence of marker positivity among the families causes an overestimate of ESBL positivity due to the presence of non-ESBL alleles. Second, the narrow range of the PCR panel causes the failure of detecting the phenotype-positive isolates possessing either unscreened or divergent ESBL genes. The negative value of kappa does not signify active disagreement but signifies a very poor agreement excluding the possibility of coincidence. The result of the exact McNemar test reveals the directional nature of this inconsistency.

The results of the ERIC-PCR analysis showed high genetic variability among *E. coli* isolates with positive markers, identifying 16 different fingerprint patterns out of the total of 35 isolates under investigation. This means that the *β-lactamase*–related markers are distributed in different genetic backgrounds rather than representing an expansion of a single clone. The same conclusion has been reached during recent genomic studies, according to which the development of resistance to extended-spectrum cephalosporins may take place in different bacteria populations through various evolutionary routes [9, 10]. However, one needs to consider the limitations of the ERIC-PCR technique while making conclusions about the findings obtained. Compared to other more advanced techniques, including core-genome SNP, MLST, or plasmid resolved WGS, ERIC-PCR is less reproducible and less discriminatory. That means that while it can be

used to show general diversity of the strains, it is not able to trace the actual transmission of bacteria and to differentiate between the spread of clones and plasmids carrying resistance genes.

Antimicrobial resistance pattern reported in the current study has important clinical relevance in Iraq. The presence of high levels of resistance against tetracycline, trimethoprim-sulfamethoxazole and quinolones indicates high antimicrobial selective pressure, which can result in the spread of multidrug-resistant bacteria. This highlights the importance of enhancing the national surveillance systems based on MIC determination, using current breakpoints, defining multidrug resistance according to standard criteria, and gathering epidemiological information on isolated pathogens. New studies demonstrate that combination of standard antimicrobial susceptibility testing and whole-genome sequencing (WGS) results in better characterization of the resistance determinants and can detect resistance genes not identified through standard phenotypic testing approaches [5, 6].

5. CONCLUSION

In the current study, the clinical isolates of Escherichia coli showed high frequency of *β-lactamase* family markers, multi-drug resistance, and high levels of genetic diversity. Based on sequencing, the major alleles included TEM-1, SHV-1, OXA-1, and DHA-1. It was therefore suggested that the detection of *β-lactamase* gene family by PCR should not be considered as proof of ESBL activity. In addition, the disparity found between the phenotypic and molecular results showed that either specific PCR methods or phenotypic assays using inhibitor could not fully characterize the *β-lactamase* resistance. Future research on *E. coli* isolates in Iraq will require incorporation of standardized susceptibility assay together with sequencing of alleles, and wherever possible genome and plasmid resolved sequencing.

6. Acknowledgements

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7. Conflicts of Interest: The Author declares that there are no conflicts of interest related to this study.

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