Iranian Journal of Microbiology

Volume 18 Number 4 (August 2026) 544-552
DOI: http://doi.org/10.18502/ijm.v18i4.22197

ORIGINAL ARTICLE

Mutation analysis of dihydrofolate reductase (*dfr*) and dihydropteroate synthase (*sul*) genes in trimethoprim-sulfamethoxazole-resistant *Escherichia coli* from urinary tract infections

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Received: April 2025**, Accepted:** July 2026

ABSTRACT

Background and Objectives: *Escherichia coli* is the leading cause of urinary tract infections (UTIs) and has shown increasing resistance to trimethoprim–sulfamethoxazole (TMP-SMX). Resistance to TMP-SMX is commonly associated with the acquisition of resistance genes such as *dfr* and *sul*, often mediated by horizontal gene transfer. This study aimed to characterize the presence of *dfr* (*dfr*A1, *dfr*A5, *dfr*A7/17) and *sul* (*sul*1 and *sul*2) genes and to describe sequence variations in selected *E. coli* isolates.

Materials and Methods: Urine culture samples were obtained from 678 patients suspected of having UTIs, and 21 samples yielded positive culture results. This study further analyzed 11 *E. coli* isolates, consisting of eight TMP-SMX-resistant isolates, two susceptible isolates, and one *E. coli* ATCC 25922 strain as a control. The clinical isolates were provided by the Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia. PCR amplification and agarose gel electrophoresis were used to identify *dfr* and *sul* genes, followed by Sanger sequencing of selected amplicons. Sequence data were analyzed with BioEdit software and aligned against reference sequences from NCBI.

Results: The distribution of resistance genes varies among resistant isolates. Some isolates carry the *dfr*A5 gene, while *dfr*A1 was detected in one isolate. The *sul*2 gene was present in a number of resistant isolates, while *sul*1 was identified but showed no differences from the reference sequence. Further sequence analysis revealed amino acid changes in the *dfr*A1, *dfr*A5, and *sul*2 genes, although these changes were not consistently found in all isolates.

Conclusion: This study characterizes the distribution of *dfr* and *sul* genes and their sequence variations in a limited number of *E. coli* isolates. The results suggest that TMP-SMX-resistant isolates may contain diverse genetic determinants associated with resistance. Nevertheless, the limited isolate number restricts the broader interpretation of these findings. Larger-scale studies, supported by functional analyses, are required to determine the contribution of these genes and their sequence variations to antibiotic resistance.

Keywords: *Escherichia coli*; Trimethoprim-sulfamethoxazole; Dihydrofolate reductase; Dihydropteroate synthase; Antibiotic resistance; Gene characterization

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INTRODUCTION

Antibiotic resistance in bacterial pathogens continues to represent a major challenge to global health (1, 2). Among pathogens associated with urinary tract infections, *Escherichia coli* is one of the most common and contributes significantly to both community-acquired and hospital-acquired infections (3, 4). Frequent antibiotic use, especially when not properly prescribed, has played a key role in the emergence of resistant *E. coli* strains, including those resistant to trimethoprim–sulfamethoxazole (TMP-SMX), a drug combination widely used for empirical urinary tract infections (UTI) therapy (5-7).

The synergistic action of trimethoprim and sulfamethoxazole stems from their ability to block successive steps in the bacterial folate biosynthesis pathway. Specifically, SMX inhibits dihydropteroate synthase (DHPS), while TMP acts downstream to target dihydrofolate reductase (DHFR) (8, 9). However, bacteria can evade this dual-drug therapy by acquiring specific resistance genes. Trimethoprim resistance is largely driven by the uptake of *dfr* genes, which encode bypass DHFR enzymes with a significantly lower affinity for the drug (10, 11). Correspondingly, SMX resistance is typically mediated by *sul* genes that produce structurally altered DHPS variants, rendering the bacteria less sensitive to inhibition (12).

The *dfr* and *sul* genes are commonly found in mobile genetic elements (MGE), including plasmids and integrons, which can promote horizontal gene transfer (HGT) among bacterial populations (13, 14). Through this process, resistance genes may spread across different bacterial strains and environments. Although chromosomal mutations can also contribute to resistance, acquired resistance genes remain an important factor in TMP-SMX resistance in *E. coli* (9).

The distribution of the *dfr* and sul genes in *E. coli* has been documented in previous studies involving isolates from clinical and environmental sources (15, 16). However, evidence regarding the presence of these genes and their sequence variations in local clinical isolates remains limited. Therefore, gene detection must be interpreted separately from sequence variation analysis, as the presence of resistance genes does not always indicate functional changes or confirm phenotypic resistance.

Therefore, this study aims to characterize the presence of selected *dfr* (*dfr*A1, *dfr*A5, *dfr*A7/17) and *sul* (*sul*1 and *sul*2) genes in *E. coli* isolates and to describe sequence variations identified through molecular analysis. This study is designed as a descriptive investigation of resistance-associated genetic elements in a limited number of isolates and does not aim to establish causal relationships or broader epidemiological conclusions. The findings are expected to provide preliminary data to support further studies with larger sample sizes and more comprehensive analytical approaches.

MATERIALS AND METHODS

Study design and ethical approval. This study was designed as a descriptive laboratory-based study to characterize resistance-associated genes in *Escherichia coli* isolates. Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. KET- 1333/UN2.FI/ETIK/PPM.00.02/2023).

Bacterial isolates. This retrospective descriptive study was conducted using urine culture samples collected in 2022 from patients with urinary tract infections (UTIs). A total of 678 urine culture samples were processed at the Microbiology Laboratory, Department of Microbiology, Faculty of Medicine, University of Indonesia, DKI Jakarta, Indonesia. Twenty-one samples tested positive in urine culture. Eleven *E. coli* isolates were selected for this study, including eight isolates with phenotypic resistance to trimethoprim–sulfamethoxazole (TMP-SMX), two susceptible isolates, and one control strain, *E. coli* ATCC 25922. All clinical isolates were obtained from urine samples of patients diagnosed with UTIs. Ethical approval for this study was granted by the Ethics Committee of Dr. Cipto Mangunkusumo National General Hospital, Faculty of Medicine, Universitas Indonesia, with approval number Kct.625/UN2.F1/ETIK/PPM.00.02/2022.

Bacterial identification and antimicrobial susceptibility testing. The *E. coli* species confirmation identification test used a Vitek® 2 Compact semi-automatic machine. The sample preparation started with fresh bacterial isolates (18-24 hours) suspended in a polystyrene tube containing 3 ml of 0.45% NaCl solution, pH 5.0, and then homogenized using a vortex. The turbidity of the inoculum was measured using a

Densicheck Plus device. In this study, the suspension turbidity used was 0.5 McFarland. From the first tube containing the inoculum with proper turbidity, 145 µl was inserted into the GN (Gram-Negative) card using a micropipette and sterile tip. The first tube was prepared for identification, and the second tube was prepared for AST (Antimicrobial Susceptibility Testing) sequentially, with appropriate cassettes for each. Incubation was carried out on a Vitek® machine for ± 24 hours. After 24 hours, identification data of the suspended isolate and information regarding the antibiotic resistance profile of the isolate were obtained (17).

DNA extraction. Genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. Briefly, bacterial colonies were suspended in phosphate-buffered saline (PBS), followed by proteinase K digestion and lysis. DNA was purified using a spin-column and eluted in 200 µL of elution buffer. Extracted DNA was stored at -20°C until further use (18).

PCR amplification of *dfr* and *sul* genes. Detection of *dfr*A1, *dfr*A5, *dfr*A7/17, *sul*1, and *sul*2 genes was performed using conventional PCR with gene-specific primers (Table 1). PCR conditions were adopted from previously published protocols and were not further optimized, as the primary objective was qualitative detection of target genes prior to sequencing.

Each PCR reaction was carried out in a final volume of 20 µL containing:

1. 10 µL 2× PCR master mix (i-Max II, iNtRON Biotechnology)
2. 1 µL forward primer (10 pmol/µL)
3. 1 µL reverse primer (10 pmol/µL)
4. 1 µL DNA template
5. 7 µL nuclease-free water

PCR amplification was performed using a thermocycler with reaction conditions that started with an initial denaturation step at 94°C for 5 minutes. After that, the amplification process continued for 30 cycles, consisting of (1) a denaturation step at 94°C for 30 seconds and (2) an annealing step at temperatures adjusted according to the target primer, namely 52°C for *dfr*A1, 55°C for *dfr*A5, 60°C for *dfr*A7/17, and 58°C for *sul*1 and *sul*2. Next, (3) an extension step was performed at 72°C for 45 seconds. After the entire cycle is complete, a (4) final extension step is performed at 72°C for 7 minutes.

Controls:

1. Positive control: previously confirmed *E. coli* isolates carrying target genes (*E. coli* ATCC 25922).
2. Negative control: PCR mixture without DNA template (no-template control).

Agarose gel electrophoresis. PCR products were analyzed using 1% agarose gel electrophoresis in 1× TAE buffer. Five microliters of PCR product were loaded into each well along with a 100 bp DNA ladder (Thermo Scientific™, GeneRuler™ 100 bp DNA Ladder, Cat. No. SM0241). Electrophoresis was performed at 100 V for 30-40 minutes. DNA bands were visualized under UV illumination and documented (18).

DNA sequencing and quality control. Selected PCR products with clear and specific amplification bands were sent to a commercial provider for Sanger sequencing. Both forward and reverse primers were used to obtain sequence data from each selected product. The quality of the raw sequences was evaluated by inspecting the chromatograms. After chromatogram examination confirms the presence of a clear single peak and minimal background interference, the sequence is included in the analysis. PCR product was purified before the sequencing process using a commercial PCR purification kit in accordance with the manufacturer's instructions (19, 20).

Sequence analysis. Sequence alignment and analysis were performed using BioEdit software. The consensus sequence was generated from the forward and reverse reads and then aligned with the reference sequence obtained from the NCBI GenBank database (21, 22). Mutation analysis was conducted at both nucleotide and amino acid levels by comparing the study sequences with reference sequences. Only consistent and high-quality sequence differences were reported as variations.

Data analysis. This study employed a descriptive analytical approach. The presence or absence of resistance genes and observed sequence variations were summarized without inferential statistical analysis due to the limited sample size.

Electrophoresis. Gel electrophoresis was performed by pouring PCR products on 1% agarose gel in 1X TAE buffer. Each well on agarose was filled with 5 µL of PCR product samples of *dfr*1, *dfr*5, *dfr*7/17,

Table 1. Primers used for *dfr* and *sul* genes (15-17)

Gene group	Primer	Sequence (5' → 3')	Amplicon size (bp)	Reference
*dfr*A1	*dfr*A1-F	TGGTAGCTATATCGAAGAATGGAGT	425	(7, 11)
	*dfr*A1-R	TATGTTAGAGGCGAAGTCTTGGGTA		(7, 11)
*dfr*A5	*dfr*A5-F	AGCTACTCTTTAAAGCCTTGACGTA	341	(7, 11)
	*dfr*A5-R	GTGTTGCTCAAAAACAACTTCG		(7, 11)
*dfr*A7/17	*dfr*A7/17-F	ACATTTGACTCTATGGGTGTTCTTC	280	(7, 11)
	*dfr*A7/17-R	AAAACTGTTCAAAAACCAAATTGAA		(7, 11)
*sul*1	*sul*1-F	CGGCGTGGGCTACCTGAACG	432	(12)
	*sul*1-R	GCCGATCGCGTGAAGTTCCG		(12)
*sul*2	*sul*2-F	GCGCTCAAGGCAGATGGCATT	293	(12)
	*sul*2-R	GCGTTTGATACCGGCACCCGT		(12)

*sul*1, and *sul*2 gene groups into each gel well. The 100 bp DNA ladder was used for markers. Then, the electrophoresis chamber was electrified at a voltage of 100 V for 30-35 minutes for the *dfr* genes, while for the *sul*1 and *sul*2 genes at a voltage of 100 V for 40 minutes. Furthermore, the amplicons were visualized using UV light (18).

Sequencing. DNA sequencing was performed using the Sanger method, which was carried out by a third party, PT Genetics Science. The sequencing procedure was performed in two reactions using forward and reverse primers. The sequencing results were edited by overlapping techniques using SecScape software to ensure correct nucleotide sequence results (19).

RESULTS

Identification and antimicrobial susceptibility testing. A total of 11 *Escherichia coli* isolates were analyzed, consisting of eight isolates resistant to trimethoprim–sulfamethoxazole (TMP-SMX), two sensitive isolates, and one reference strain (*E. coli* ATCC 25922). All isolates were confirmed as *E. coli* using the Vitek® 2 Compact system. Phenotypic susceptibility testing showed that isolates numbered 10, 14, 29, 53, 78, 79, 88, and 95 were resistant to TMP-SMX, while isolates 73 and 80, as well as the ATCC control strain, were sensitive. The detailed results of identification and gene detection are summarized in Table 2.

Detection of *dfr* and *sul* Genes by PCR. PCR amplification demonstrated variability in the presence of resistance genes among the isolates (Table 2). Not all resistant isolates carried detectable *dfr* or *sul* genes. The *dfr*A1 gene was detected only in isolate 95, as indicated by a DNA band at 425 bp (Fig. 1). No amplification was observed in sensitive isolates (73 and 80) or in the control strain.

The *dfr*A5 gene was detected in isolates 14, 53, 78, 88, and 95, with a DNA band at 341 bp (Fig. 2). No bands were observed in sensitive isolates or the control strain. The *dfr*A7/17 gene was identified in isolates 29, 79, and 95, with a DNA band at 280 bp (Fig. 3). The control strain did not show amplification. The results of the *sul*1 target gene showed DNA bands at 432 bp

Table 2. Identification and sensitivity test of *E. coli* using Vitek and PCR.

Isolate	Phenotype (Vitek)	*dfr*A1	*dfr*A5	*dfr*A7/17	*sul*1	*sul*2
10	Resistant	–	–	–	–	–
14	Resistant	–	+	–	–	+
29	Resistant	–	–	+	+	+
53	Resistant	–	+	–	–	–
78	Resistant	–	+	–	+	+
79	Resistant	–	–	+	+	+
88	Resistant	–	+	–	–	+
95	Resistant	+	+	+	+	–
73	Sensitive	–	–	–	–	–
80	Sensitive	–	–	–	–	–
ATCC 25922	Sensitive control	–	–	–	–	–

*Resistant to the antibiotic trimethoprim/sulfamethoxazole; S: sensitive; (+): Bands appear on agarose gel; (-): no bands appear on agarose.

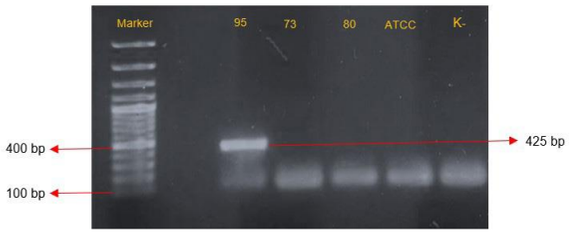

Fig. 1. DNA band amplified by the *dfr*1 gene in *E. coli*. Resistant isolates (95); sensitive isolates (73 and 80); ATCC: *E. coli* ATCC 25922 (control); K-: negative control.

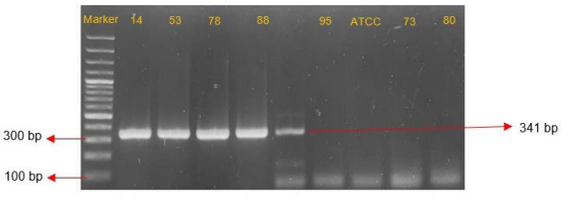

Fig. 2. DNA band amplified by the *dfr*5 gene in *E. coli*. Resistant isolates (14, 53, 78, 88, and 95); sensitive isolates (73 and 80); ATCC: *E. coli* ATCC 25922 (control); sensitive isolate (80).

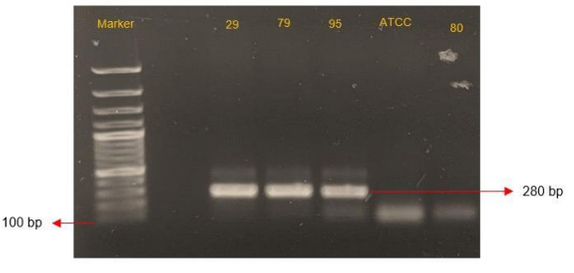

Fig. 3. DNA band amplified by *dfr*7&17 genes in *E. coli*. M: DNA ladder from bottom to top is 100 bp -1400 bp; resistant isolates (29, 79, and 95); ATCC: *E. coli* ATCC 25922 (control); sensitive isolate (80).

in isolates 29, 78, 79, and 95, consistent with Table 2 and Fig. 4. The *sul*2 gene was detected in isolates 14, 29, 78, 79, and 88, with a DNA band at 293 bp (Fig. 5). No amplification was observed in sensitive isolates or the control strain.

PCR electrophoresis analysis showed that the distribution of resistance-associated genes varied among clinical *E. coli* isolates. The *dfr* gene was detected in several isolates resistant to trimethoprim, while *sul*1 and *sul*2 were detected in isolates associated with sulfamethoxazole resistance. The bands at the expected amplicon sizes support the amplification of the target genes and suggest that TMP-SMX resistance in these

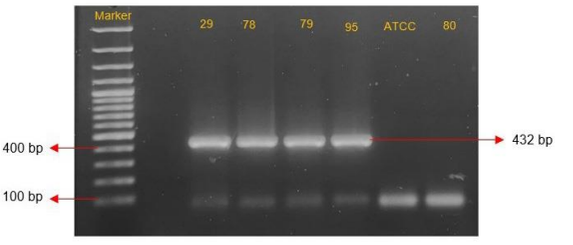

Fig. 4. DNA band amplified by *sul*1 gene in *E. coli*. M: DNA ladder from bottom to top is 1100 bp -1400 bp; resistant isolates (29, 78, 79, and 95); ATCC: *E. coli* ATCC 25922 (control); sensitive isolate (80).

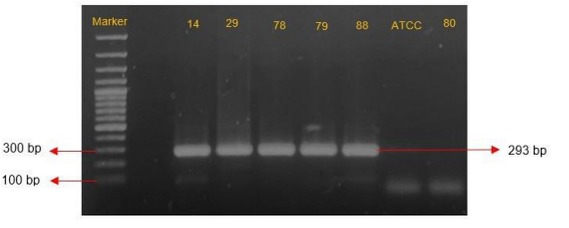

Fig. 5. DNA band amplified by *sul*2 gene in *E. coli*. M: DNA ladder from bottom to top is 100 bp -1400 bp. Resistant isolates (14, 29, 78, 79, and 88); ATCC: *E. coli* ATCC 25922 (control); sensitive isolate (80).

isolates may involve different combinations of the *dfr* and *sul* genes. However, it is important to note that these resistant isolates do not share a similar genetic profile, indicating molecular heterogeneity in their resistance mechanisms. Nevertheless, these PCR results should be interpreted as preliminary molecular evidence, since agarose gel electrophoresis does confirm the presence of amplicons of the expected size but does not determine the exact sequence identity or functional effects of the detected genes.

DISCUSSION

Antimicrobial resistance in *Escherichia coli* still stands as a significant problem in urinary tract infections, particularly because UPEC remains the leading cause of UTIs and is showing increasing resistance to commonly used antibiotics (20-23). In this study, TMP-SMX-resistant *E. coli* isolates from UTI patients were analyzed to detect *dfr* and *sul* genes and to describe the amino acid changes associated with these genes. The results showed that resistance determinants were distributed differently among the isolates, indicating that TMP-SMX resistance in

clinical *E. coli* may arise from varied genetic backgrounds and different resistance mechanisms.

The detection of the *dfr* gene in several isolates is consistent with known mechanisms of trimethoprim resistance. Generally, this resistance occurs when bacteria acquire the *dfr*A gene, which encodes an alternative dihydrofolate reductase (DHFR) enzyme, thereby making them less sensitive to inhibition by trimethoprim (24, 25). Recent reports indicate that the *dfr*A gene is highly variable, with many variants found in Gram-negative bacteria. For this reason, sequence analysis must be interpreted with caution and supported by a standardized numbering system (24). The identification of a new *dfr*A gene in a phage plasmid also suggests that trimethoprim resistance determinants continue to evolve and can be transferred via MGE (25).

Similarly, the presence of *sul* genes in many isolates is consistent with the known mechanism of sulfonamide resistance. Sulfonamide resistance is commonly mediated by *sul*1 and *sul*2, which encode altered dihydropteroate synthase (DHPS) enzymes with reduced affinity for sulfonamides (26-28). Since TMP-SMX inhibits sequential steps in the folate biosynthesis pathway, the coexistence of *dfr* and *sul* genes provides a molecular basis for resistance to this antibiotic combination. However, the heterogeneous distribution of these genes also indicates that resistance cannot be explained by a single determinant alone, but may result from different combinations of resistance genes and additional resistance mechanisms.

The distribution pattern of *sul* genes in this study showed that *sul*2 was detected more frequently than *sul*1. This finding is consistent with recent reports on UPEC and multidrug-resistant *E. coli,* which showed that *sul*2 is frequently carried by resistant isolates, while *sul*1 is often associated with class 1 integrons (26, 29). The association of *sul*1 with class 1 integrons may explain its variable distribution among isolates, because its presence depends on the genetic background and acquisition history of integron-associated resistance elements (27, 29, 30).

In contrast, *sul*2 is frequently reported in resistant UPEC isolates and may be carried together with other antimicrobial resistance genes, supporting its role as an important determinant of sulfonamide resistance in clinical *E. coli* (26, 29). Therefore, the higher detection of *sul*2 in this study may reflect its wider distribution among TMP-SMX-resistant isolates compared with *sul*1.

Sequence analysis revealed amino acid substitutions in the *dfr*A1 gene. In this study, isolate 95 showed five amino acid substitutions, namely I55V, D64S, N65S, I70V, and N129S. Variability among *dfr*A genes has been widely reported, and recent work has highlighted the complexity of *dfr*A classification due to the large number of variants and inconsistencies in public sequence databases (24). Moreover, the recent discovery of novel *dfr*A50 and *dfr*A51 genes indicates that genetic variation in this gene group remains actively documented (25).

These substitutions may suggest the presence of sequence variation in the DHFR enzyme. However, this study cannot confirm whether such changes have a direct functional effect. Because functional assays were not performed, the substitutions identified in this study should be regarded as sequence variations, not as definitive evidence of increased trimethoprim resistance.

The *dfr*A5 gene showed consistent amino acid substitutions across several isolates, including A17G, A20S, D21N, D22D, I26V, P29Q, Y36D, Y37D, L41F, and D43G. The repeated identification of these substitutions suggests similar sequence patterns among the isolates analyzed in this study. This finding is relevant to the study by Asmare et al., which reported that *dfr*A genes in co-trimoxazole-resistant UPEC were commonly located within integrons, transposons, or single-gene cassette structures associated with MGE (26).

Research on multidrug-resistant *E. coli* has also reported that integron-associated resistance cassettes can occur in various combinations, which may contribute to multidrug resistance (29). In this context, the *dfr*A5 substitution identified in this study should not be overinterpreted at this stage. Instead, it should be viewed as a genetic variation that requires further validation in terms of genetic context mapping and phenotypic validation.

In the analysis of the *sul* gene, *sul*1 does not show any amino acid changes compared to the reference sequence KF240816.1. This indicates that the *sul*1 sequence is relatively conserved across all analyzed isolates. This finding is consistent with previous studies reporting that *sul*1 is frequently carried in class 1 integrons, particularly in conserved regions that support the transfer of resistance gene cassettes (27, 29). The absence of amino acid substitutions may also indicate that sulfonamide resistance associated

with *sul*1 can occur without additional sequence variations in the analyzed region.

Amino acid substitutions at Gly8Trp and Ile12Met were observed in several *sul*2 isolates, a feature not found in *sul*1. The fact that *sul*2 consistently appears in UPEC strains resistant to various drugs, particularly in conjunction with additional resistance genes, further reinforces its involvement in antifolate resistance (31). However, the specific functional consequences of the Gly8Trp and Ile12Met mutations still remain an open issue. Without empirical support from enzymatic assays, structural analysis, or MIC data, these variations should be regarded as basic natural polymorphisms, local sequence shifts, or isolate-specific adaptations.

Despite its resistance to TMP-SMX, isolate 10 showed no amplification of the targeted *dfr* or *sul* genes. This finding suggests that the resistant phenotype may be associated with alternative mechanisms, or with *dfr/sul* variants that were not covered by the primer sets used in this study. TMP-SMX resistance in *E. coli* is not limited to the acquisition of *dfr*A and *sul* genes. Other mechanisms, including chromosomal alterations in folate pathway genes, reduced membrane permeability, enhanced efflux pump activity, biofilm formation, adaptive bacterial responses, or other unexplored resistance determinants, may also contribute to resistance (31, 32). Accordingly, the resistance observed in isolate 10 cannot be fully attributed to the absence of the targeted genes. Further investigation using broader molecular methods, including whole-genome sequencing, would be valuable to identify other possible resistance mechanisms.

The genetic background of the detected resistance genes was not further investigated in this study. As a result, their association with plasmids, transposons, integrons, or other MGE could not be determined. This represents an important limitation, as recent evidence shows that *dfr*A and *sul* genes are often carried by class 1 or class 2 integrons, transposons, gene cassettes, plasmids, or other mobile structures involved in horizontal gene transfer (28, 30, 33).

The lack of plasmid profiling, integron characterization, and whole-genome sequencing limited this study's ability to identify whether the detected genes were carried by transferable genetic elements. Accordingly, conclusions regarding gene mobility, clonal relationships among isolates, and epidemiological spread should be interpreted with caution. Further research should examine the genetic context of these resistance genes to determine their potential association with transferable resistance elements (34).

This study has several limitations. First, the number of isolates analyzed was relatively small, which limits the extent to which these findings can be generalized to a broader clinical population of *E. coli*. Second, the analysis focused only on specific resistance genes, while other potential resistance determinants and chromosomal mutations were not investigated. Furthermore, no functional assays were performed. Therefore, the biological relevance of the identified amino acid substitutions cannot be confirmed. Since this study employed a descriptive design, the findings should not be interpreted as evidence of a cause-and-effect relationship with the underlying resistance mechanisms (35).

PCR amplification may have been affected by poor optimization, particularly for the *dfr*7 and *dfr*17 genes. Sequencing results supported the specificity of the PCR assays used in this study. Although the findings should be considered preliminary molecular data rather than definitive evidence of the resistance mechanism, they provide useful baseline information on the distribution of *dfr* and *sul* genes in clinical TMP-SMX-resistant *E. coli* isolates from UTI cases. The higher detection of *sul*2 compared with *sul*1, together with nonsynonymous amino acid substitutions in *dfr*A1, *dfr*A5, and *sul*2, suggests genetic variation among these antifolate resistance determinants (35).

This study demonstrates that TMP-SMX resistance in clinical *E. coli* extends beyond simple gene acquisition, involving complex sequence variations and unresolved background mechanisms. These findings need to be explored further using a larger number of isolates and broader molecular analysis. Future research should consider whole-genome sequencing, plasmid mapping, resistome analysis, protein structure prediction, and functional assays to clarify the role of the detected variants. This information may help improve antimicrobial surveillance and support better antibiotic use in the management of UTIs (36).

CONCLUSION

This study examined TMP-SMX-resistant *Escherichia coli* isolates from urinary tract infection cases to identify the presence of *dfr* and *sul* genes and de-

scribe their related amino acid changes. The results showed that resistance determinants were not distributed uniformly among the isolates. Several mutations were similar to variants reported in previous studies, while some sequence patterns may represent genetic variations found in the local isolates. The detection of *dfr*A1, *dfr*A5, and *sul*2 suggests that horizontally acquired resistance genes play an important role in TMP-SMX resistance. Nevertheless, one resistant isolate did not contain the targeted *dfr* or *sul* genes, which indicates that other resistance mechanisms may be involved but were not explored in this study. In general, these findings provide early molecular information on TMP-SMX resistance in clinical *E. coli* isolates. Because this study used a limited number of isolates and focused only on selected genes, further research with larger samples and whole-genome sequencing is needed to better clarify the genetic diversity and clinical importance of these resistance determinants.

ACKNOWLEDGEMENTS

The authors are grateful to the Clinical Microbiology Laboratory, Department of Microbiology, Faculty of Medicine, University of Indonesia, for providing the *E. coli* isolates used in this study. There are no conflicts of interest related to this study. This study was supported by grant *Publikasi Terindeks International* (PUTI), 2022.

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