

RESEARCH ARTICLE

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Identification of ESBL-producing *Escherichia coli* Carrying Genes Coding Aminoglycosides Modifying Enzymes Isolated from Urinary Tract Infection Patients in Sana'a City, Yemen

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Abstract

Resistance to antibiotics poses a major challenge, especially in cases of urinary tract infections attributed to *Escherichia coli*. The rise of extended-spectrum β -lactamase (ESBL)-producing and aminoglycoside-resistant strains complicates treatment. This research focused on identifying the occurrence of both phenotypically confirmed ESBL and AME genes in *E. coli* isolated from patients with UTIs in Sana'a, Yemen. This cross-sectional study encompassed a sample of 378 patients at Al-Kuwait University Hospital, Sana'a. Midstream urine samples were cultured and *E. coli* isolates identified via standard methods. Susceptibility to antimicrobial agents was determined through the Kirby-Bauer method, following the guidelines issued by CLSI in 2019. ESBL production was phenotypically detected and AME genes (*aac*(6')-Ib, *aac*(3')-IIa, *aph*(3')-Ia, and *ant*(2'')-Ia) were detected via multiplex PCR. Among 378 samples, 167 *E. coli* isolates were identified (44.18%), of which 76 (45.5%) were ESBL-producers. AME genes were detected in 52.6% of ESBL isolates, with *aac*(6')-Ib being most frequent (39.47%), followed by *ant*(2'')-Ia (31.58%) and *aph*(3')-Ia (11.84%). Co-occurrence of ≥ 2 AME genes was seen in 36.4% of isolates. Risk factors for resistance included catheterization, hospitalization, and older age. The study identified a high rate of dual antimicrobial resistance among *E. coli* isolates in Yemen underscores the need for enhanced molecular surveillance and antimicrobial stewardship programs. Empiric therapies should prioritize amikacin and carbapenems in high-risk cases.

Keywords: *Escherichia coli*, ESBL, Aminoglycoside-Modifying Enzymes, Antimicrobial Resistance, Urinary Tract Infection, Yemen

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INTRODUCTION

The increasing prevalence of antibiotic-resistant pathogens, particularly multidrug-resistant *E. coli*, significantly complicates the treatment of everyday infections like UTIs, both outside and within healthcare facilities.¹ UTIs, predominantly caused by Enterobacteriaceae such as *E. coli*, account for 40% of hospital-acquired infections and are increasingly complicated by resistance to first-line therapies.^{2,3} Expansion of *E. coli* strains harboring extended-spectrum β -lactamase (ESBL) determinant, which hydrolyze cephalosporins and monobactams, has exacerbated this crisis, with resistance rates exceeding 40% in regions like the Middle East.^{4,5} Risk factors such as prolonged hospitalization, invasive procedures, and antibiotic overuse further drive resistance, limiting therapeutic options.^{6,7}

ESBLs, encoded by mobile genetic elements such as plasmids, confer resistance to β -lactams through enzymatic inactivation.⁸ ESBL-producing *E. coli* often display combined to other drug classes, including aminoglycosides and fluoroquinolones, due to the clustering of resistance genes on shared plasmids.^{9,10} Carbapenems remain the last-resort treatment, but rising resistance underscores the urgency for alternative strategies.¹¹⁻¹³

Aminoglycosides, such as gentamicin and amikacin, are potent bactericidal agents used against Gram-negative infections, particularly in critically ill patients.^{14,15} However, their nephrotoxicity and ototoxicity limit prolonged use, while resistance mediated by aminoglycoside-modifying enzymes (AMEs) has surged globally.¹⁶⁻¹⁹ AMEs, including acetyltransferases (AACs) and phosphotransferases (APHs), inactivate aminoglycosides by structural modification, with genes like *aac(6')-Ib* and *ant(2'')-Ia* frequently linked to ESBL plasmids.¹⁹⁻²¹ This co-resistance complicates treatment regimens and highlights the need for molecular surveillance.²²

Antimicrobial resistance, ranked fifth among global health threats by WHO in 2019, drives cross-resistance and complicates treatment in vulnerable populations.²³ The genetic co-localization of ESBL and AME genes on plasmids facilitates the spread of multidrug resistance, creating a significant public health burden.^{24,25}

Studies report high rates of co-resistance in regions with unregulated antibiotic use, such as Iran (41%) and Saudi Arabia (44.8%), driven by horizontal gene transfer.^{26,27} Risk factors like hospitalization, catheterization, and comorbidities (e.g., diabetes) further amplify resistance.^{28,29} Despite global data, gaps persist in low-resource settings like Yemen, where surveillance and stewardship programs are limited.³⁰

This study addresses the paucity of data on ESBL and aminoglycoside co-resistance in Yemen, focusing on *E. coli* isolates from UTIs in Sana'a. It aims to (1) assess the frequency of ESBL-producing *Escherichia coli* isolates, (2) identify predominant AME genes mentioned in Table 1, and (3) evaluate risk factors for co-resistance (e.g., prior hospitalization, antibiotic exposure). By characterizing resistance patterns and genetic mechanisms, this work seeks to inform local treatment guidelines and stewardship efforts, challenging the hypothesis that no association exists between AME genes and ESBL production in Yemeni UTI patients.

Although reports from various regions highlight the frequent co-detection of AME and ESBL genes, there is a notable lack of such data from Yemen. The present study is the first conducted in Sana'a to assess the co-occurrence of these resistance genes in *E. coli* isolates from individuals diagnosed with urinary tract infections.

MATERIALS AND METHODS

The design of the research

The study adopted a cross-sectional design and included patients attending either the outpatient clinic or inpatient wards of Al-Kuwait University Hospital in Sana'a, Yemen. The study was approved by the Ethics Committee, Faculty of Medicine and Health Sciences, Sana'a University, Republic of Yemen, and informed consent was obtained from the participants before enrolling in the study. A total of 378 urine specimens were collected from participants of both sexes and varying ages presenting with symptoms consistent with urinary tract infection (UTI). Individuals who had used antibiotics in the preceding two days or had contaminated samples were not included in the analysis.

Table 1. Primer sequences used for detection of Aminoglycoside-Modifying Enzyme (AME) genes

Target gene	Primer Sequence (5'-3')	Size of amplified product (bp)
aac(3')-IIa	F 5'-ATGCATACGCGGAAGGC-3'	822 bp
	R 5'-TGCTGGCACGATCGGAG-3'	
aac(6')-Ib	F 5'-TATGAGTGGCTAAATCGAT-3'	395 bp
	R 5'-CCCGCTTCTCTAGCA-3'	
ant(2'')-Ia	F 5'-ATCTGCCGCTCTGGAT-3'	404 bp
	R 5'-CGAGCCTGTAGGACT-3'	
aph(3')-Ia	F 5'-CGAGCATCAAATGAAACTGC-3'	623 bp
	R 5'-GCGTTGCCAATGATGTTACAG-3'	

Sample collection and culture

Sterile, wide-mouthed containers were used to collect clean-catch midstream urine samples under aseptic conditions. All samples were processed within two hours of collection. Each specimen was inoculated onto Blood Agar and MacConkey Agar using a calibrated loop equivalent to 0.001 mL. Incubation was performed at 37 °C for 24 hours. A bacterial load of $\geq 10^5$ CFU/mL was considered diagnostic of infection, and only one isolate per individual was included in the analysis.

Identification of isolates

Bacterial isolates were preliminarily detected by Gram staining and colony morphology, followed by biochemical identification using standard tests including indole, citrate utilization, urease production, triple sugar iron agar, and oxidase. Confirmatory identification was done by using the Microscan WalkAway Plus 96 automated system.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was carried out using the modified Kirby-Bauer disk diffusion technique, following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2019). The tested antimicrobial agents included aminoglycosides such as gentamicin, amikacin, and tobramycin, as well as β -lactam antibiotics including ceftazidime, cefotaxime, and cefepime. Interpretation of inhibition zones was based on CLSI-established breakpoints.

Phenotypic detection of ESBL

ESBL activity was assessed using the double-disk synergy test using ceftazidime and cefotaxime disks, both alone and in combination with clavulanic acid. An increase of ≥ 5 mm in the zone of inhibition with clavulanate compared to ceftazidime or cefotaxime alone indicated ESBL production. Additionally, automated confirmation was obtained using the Microscan system.

DNA extraction and PCR for AME genes detection

Genomic DNA was purified from bacterial colonies using AccuPrep Genomic DNA Extraction, Bioneer, Korea, following the instructions provided by the manufacturer. Multiplex PCR assays were performed using gene-specific primers synthesized by Microsynth AG - Balgach, Switzerland, and the Primer nucleotide sequences and expected sizes of amplicons for the targeted genes are presented in Table 1. Each Primer diluted according to volume indicated in the technical datasheet from Microsynth AG company. PCR products were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and visualized under UV transillumination.

DNA Sequencing

Purified PCR products were initially submitted to Genome Medical Company (Amman, Jordan) for sequencing services. The samples were then forwarded to MacroGen Europe (Milan, Italy) for Sanger sequencing. Sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl DNA Analyzer (Applied Biosystems). Both forward

Table 2. Association of risk factors among ESBL and Non-ESBL *E. coli*

Risk factors		Non-ESBL <i>E. coli</i>		ESBL <i>E. coli</i>		OR	CI Lower-Upper	χ^2	P-value
		No.	%	No.	%				
Older age	Yes	28	30.7	48	63.1	3.86	2.02-7.35	17.51	<0.001
	No	63	69.3	28	36.9				
Recent Catheterization	Yes	14	15.3	29	38.1	3.39	1.63-7.05	11.24	0.0008
	No	77	84.7	47	61.9				
Diabetic	Yes	11	12	27	35.5	4.01	1.83-8.81	12.94	0.0003
	No	80	88	49	64.5				
Kidney Disorder	Yes	5	5.4	18	23.6	5.34	1.88-15.18	11.55	0.0007
	No	86	94.6	58	76.4				
Calculi	Yes	6	6.5	14	18.4	3.20	1.17-8.78	5.49	0.019
	No	85	93.5	62	81.6				
Hospitalization/ICU	Yes	3	3.3	17	22.3	8.45	2.37-30.12	14.28	0.00016
	No	88	96.7	59	77.7				
Total		91	100%	76	100%				

and reverse sequencing reactions were carried out using the respective primers to ensure high accuracy and full coverage of the target genes.

Analysis and Deposition of DNA Sequences in GenBank

The nucleotide sequences were deposited in the GenBank/EMBL/DDBJ database. Each sequence was analyzed using the BLASTn algorithm against the NCBI database to determine percentage similarity with existing sequences. Accession numbers for the sequences generated in this study were retrieved from GenBank.

RESULTS

Culture and identification of isolates

Of the 378 urine samples analyzed, 274 (72.5%) exhibited significant bacterial growth, with *Escherichia coli* being the most frequently isolated pathogen (167 isolates, 44.18%). Other organisms included *Klebsiella* spp. (17.46%), *Pseudomonas* spp. (6.61%), and *Staphylococcus saprophyticus* (1.1%). Elderly patients (≥ 60 years) represented 27.8% of culture-positive cases, with burning urination (79.2%), fever (52.5%), and dysuria (51.5%) as predominant symptoms.

Demographic and clinical characteristics

The prevalence of culture-positive UTIs was higher among elderly patients (≥ 60 years) representing 27.8% of positive cases. The most commonly reported symptom was burning urination (79.2%), followed by fever (52.5%), dysuria (51.5%), and flank pain (48.9%).

Prevalence and risk factors for ESBL-producing *E. coli*

Among the 167 *E. coli* isolates, 76 (45.5%) were confirmed as ESBL producers. Statistical analysis identified hospitalization/ICU admission (OR = 8.45, 95% CI: 2.37-30.12, $p < 0.001$), kidney disorders (OR = 5.34, 95% CI: 1.88-15.18, $p = 0.0007$), and diabetes (OR = 4.01, 95% CI: 1.83-8.81, $p = 0.0003$) as significant risk factors (Table 2).

Antimicrobial resistance profile

ESBL-producing isolates exhibited near-universal resistance to β -lactams, including ampicillin (100%), cefotaxime (100%), and cefepime (100%). In contrast, carbapenems such as meropenem (81.6% susceptibility) and aminoglycosides like amikacin (85.5% susceptibility) demonstrated retained efficacy (Table 3).

Table 3. Distribution of ame genes among ESBL-producing *E. coli* isolates in Sana'a, Yemen

Family	Antibiotics	ESBL Uropathogenic <i>E. coli</i>						Total
		S	percent	M	percent	R	percent	
Penicillin	Ampicillin	1	1.3	0	0	75	98.7	76
	Piperacillin	5	6.6	3	3.95	68	89.47	76
Antibiotic with β -lactamase inhibitors	Ampicillin-sulbactam	17	22.4	12	15.8	47	61.8	76
	Piperacillin-tazobactam	33	43.4	11	14.47	32	42.11	76
	Amoxicillin-clavulanic acid	34	44.7	3	3.9	39	51.3	76
Monobactam	Aztreonam	3	3.9	1	1.3	72	94.7	76
Cephalosporins-2 nd Generation	Cefoxitin	28	36.8	8	10.5	40	52.6	76
	Cefuroxime	2	2.6	0	0	74	97.4	76
Cephalosporins-3 rd Generation	Cefotaxime	1	1.3	0	0	75	98.7	76
	Ceftazidime	2	2.6	1	1.3	73	96.1	76
Cephalosporins-4 th Generation	Cefepime (Cefepime)	2	2.6	1	1.3	73	96.1	76
Tetracyclines	Tetracycline	30	39.5	2	2.63	44	57.89	76
	Tigecycline	51	67.1	2	2.63	23	30.2	76
Carbapenems	Ertapenem (Ertapenem)	54	71.1	8	10.5	14	18.4	76
	Imipenem	57	75	6	7.89	13	17.11	76
	Meropenem	62	81.6	1	1.32	13	17.11	76
Fluoroquinolones	Ciprofloxacin	27	35.5	5	6.6	44	57.9	76
Amino-glycosides	Amikacin	65	85.5	3	3.9	8	10.5	76
	Gentamicin	50	65.8	6	7.89	20	26.32	76
	Tobramycin	45	59.2	5	6.58	26	34.21	76

Risk factors for AME gene carriage

Genetically confirmed aminoglycoside resistance was significantly associated recent catheterization ($p < 0.001$) as illustrated in Table 4.

Genotype-Phenotype Correlations for Aminoglycoside Resistance in ESBL-Producing *E. coli*

Of the AMG-modifying genes detected in ESBL-producing *E. coli* isolates, the presence of *aac*(6')-Ib was strongly associated with a resistant phenotype to gentamicin (70%) and tobramycin (66.7%), but not to amikacin. Similarly, *ant*(2'')-Ia correlated with resistance to tobramycin (66.7%) and, to a lesser extent, gentamicin (54.2%). The less frequent genes *aph*(3')-Ia and *aac*(3')-IIa showed distinct profiles, with *aph*(3')-Ia conferring high-level gentamicin resistance (77.8%) and *aac*(3')-IIa being linked to universal gentamicin resistance (100%) as shown in Table 5.

Similarity of DNA sequences of AME-genes produced by ESBL *E. coli*

Sequences of each resistance gene of the clinical pathogenic isolates have been deposited in GenBank to determine the similarity between the sequences of the present study and that previously deposited in GenBank by other investigators for the same genes.

Regarding the nucleotide sequence of the amplified *acc*(6')-Ib gene, it was found that 45 bacterial sequences of aminoglycoside (6) N-acetyltransferase of different bacterial species were similar by 100% with *acc*(6')-Ib gene of *E. coli*. Among these genes, aminoglycoside (6)N-acetyltransferase of *Shigella sonnei* with accession no. LC310955.1. and of *E. coli* with accession no. MF495900.

The nucleotide sequence of the *ant*(2'')-Ia gene from *Escherichia coli* exhibited 99%-100% identity to a similar gene from 39 various bacterial strains producing the same gene, among these

genes, aminoglycoside nucleotidyltransferase ant (2'')-Ia of *Pseudomonas aeruginosa* and *E. coli* isolate (accession no. NG_052369.1 and MF495900, respectively).

The sequence of aph(3')-Ia gene of *E. coli* of the present study revealed that this gene was similar by 99% with 10 different bacterial strains having aph(3')-Ia genes. Among these genes, aminoglycoside (3')phosphotransferase of *Klebsiella pneumonia* with accession no. MF495903 and *E. coli* strain P225M with accession number CP044348.1.

The sequence of acc (3')-IIa gene of *E. coli* was similar by 99% to aminoglycoside (3) N-acetyl transferase of *Klebsiella pneumonia* with accession no. PV023000.1 and *E. coli* strain AVS0970 with accession no. CP124503.1.

Determination of AME Gene Accession Numbers

The nucleotide sequences of the four AME genes identified in this study were deposited in GenBank. Sequence similarity was confirmed via BLASTn analysis against the NCBI database, revealing 99%-100% identity with previously

Table 4. Correlation between risk factors and AME gene-mediated resistance in ESBL-producing *E. coli*

Risk factors		ESBL-producing <i>E. coli</i> with AMG resistance		ESBL-producing <i>E. coli</i> without AMG resistance		OR	CI		χ^2	P-value
		(phenotypic or genotypic)		(phenotypic or genotypic)			Lower	Upper		
		No.	%	No	%					
Older age	Yes	28	63.6	20	62.5	1.1	0.41	2.7	0.01	0.919
	No	16	36.4	12	37.5					
Recent Catheterization	Yes	28	63.6	1	3.1	54.2	6.8	435.9	28.7	<0.001
	No	16	36.4	31	96.9					
Diabetic	Yes	17	38.6	10	31.3	1.4	0.53	3.6	0.44	0.507
	No	27	61.4	22	68.8					
Kidney Disorder	Yes	9	20.5	9	28.1	0.66	0.23	1.9	0.6	0.437
	No	35	79.5	23	71.9					
Calculi	Yes	9	20.5	5	15.6	1.4	0.42	4.6	0.29	0.592
	No	35	79.5	27	84.4					
Hospitalization/ICU	Yes	8	18.2	9	28.1	0.57	0.19	1.7	1.1	0.304
	No	36	81.8	23	71.9					
Total		44	100	32	100	76				

Table 5. Genotype-Phenotype Correlations for Aminoglycoside Resistance in ESBL-Producing *E. coli*

Phenotypically resistance/ Genetically resistance		Phenotypically resistance					
		Amikacin		Gentamycin		Tobramycin	
		Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
AMG encoding	aac(6')-Ib	22 (53.3%)	8 (26.7%)	9 (30%)	21 (70%)	10 (33.3%)	20 (66.7%)
genes among	ant(2'')-Ia	16 (66.7%)	8 (33.3%)	11 (45.8%)	13 (54.2%)	8 (33.3%)	16 (66.7%)
ESBL-producing	aph(3')-Ia	7 (77.8%)	2 (22.2%)	2 (22.2%)	7 (77.8%)	5 (55.6%)	4 (44.4%)
<i>E. coli</i> isolates	aac(3')-IIa	1 (33.3%)	2 (66.7%)	0 (0%)	3 (100%)	1 (33.3%)	2 (66.7%)

Table 6. Determination of accession numbers by GenBank

Sequence_ID	Identifier Organism GenBank accession number
Seq-1: aac(6')-Ib	<i>Escherichia coli</i> PX219619
Seq-2: ant(2'')-Ia	<i>Escherichia coli</i> PX219620
Seq-3: aph(3')-Ia	<i>Escherichia coli</i> PX219621
Seq-4: aac(3')-IIa	<i>Escherichia coli</i> PX219618

reported homologs. Unique accession numbers were assigned to each gene, as listed in Table 6.

DISCUSSION

The findings indicate a high prevalence of multidrug-resistant (MDR) *Escherichia coli* among UTI patients in Sana'a, Yemen. Nearly half of the *E. coli* isolates were ESBL producers, and over half of these showed concurrent resistance to aminoglycosides. These findings align with regional trends, reinforcing *E. coli* as the leading uropathogen with increasing resistance rates.

The 45.5% prevalence of ESBL-producing *E. coli* observed here is comparable to reports from Egypt (44.6%), Iran (41%), and Pakistan (40%).⁴⁻⁶ However, higher rates have been reported in Jordan, Syria (50%-62%),⁷ while lower rates were seen in parts of Saudi Arabia (27.4%-33%).⁸ These differences likely reflect variability in antimicrobial stewardship, diagnostic capacity, and healthcare infrastructure.

Our results show universal resistance of ESBL-producing isolates to penicillins and third-generation cephalosporins similar to patterns observed in Egypt, Iran, and Saudi Arabia.⁹⁻¹¹ In contrast, carbapenems and amikacin remained the most effective agents, making them essential for empirical treatment in high-risk cases similar to results reported by a study conducted in Pakistan.¹²

Notably, 60.5% of ESBL-producing isolates showed phenotypic resistance to aminoglycosides. PCR analysis confirmed that 52.6% carried at least one AME gene, with aac(6')-Ib being the most prevalent. These findings are in line with studies from Sudan, Egypt, and Spain.¹³⁻¹⁵ However, some studies, such as those from Tunisia and Tehran, found aac(3')-IIa to be more common,^{16,17}

suggesting regional variability in resistance gene profiles. Another study conducted in Iran reported that the most frequent AME-related genes were ant(2'')-Ia and aph(3')-Ia, followed by aac(3')-IIa.¹⁸

A strong genotype-phenotype correlation was observed, especially with aac(6')-Ib, ant(2'')-Ia and aac(3')-IIa and tobramycin/gentamicin resistance. These findings corroborate global data from Switzerland and the Middle East,^{17,18} further highlighting the diagnostic value of molecular assays in resistance surveillance.

Interestingly, some isolates (7.9%) demonstrated phenotypic resistance without detectable AME genes, suggesting alternative mechanisms such as 16S rRNA methylation or efflux pumps. Further genomic studies are warranted to elucidate these mechanisms.¹⁹

Our risk factor analysis showed that only catheterization was independently associated with both ESBL and AME gene carriage. This pattern is supported by data from China, Sierra Leone, and France,²⁰⁻²³ underscoring the role of healthcare exposure in promoting resistance. Interestingly, comorbidities like diabetes and kidney disorders were not significantly associated with AME gene carriage in our cohort, unlike findings from other studies.²⁴

All sequences of the detected amplicons were aligned and showed 99%-100% identity with the reported target genes accessed from NCBI. Similar results were obtained in studies from Egypt and Spain.^{10,31} The observed sequence homology across distinct bacterial species strongly suggests the occurrence of natural horizontal gene transfer of these resistance genes.

This study has several limitations. First, the sample size, while sufficient for initial analysis, may not fully capture the diversity of resistance patterns across different populations. Second, the study focused on specific AME genes (aac(6')-Ib, aac(3')-IIa, aph(3')-Ia, ant(2'')-Ia), and did not investigate other resistance mechanisms such as 16S rRNA methyltransferases or efflux pumps, which may contribute to aminoglycoside resistance. Finally, whole-genome sequencing was not performed, which limits the ability to track plasmid-mediated gene transfer or clonal spread.

To the best of our knowledge, this is the first study in Yemen to report the co-occurrence of ESBL production and AME genes particularly *aac(6')-Ib* in uropathogenic *E. coli*.

CONCLUSION

These findings highlight an emerging threat of multidrug-resistance in a resource-limited setting and underscore the urgent need for national antimicrobial resistance surveillance and stewardship programs in Yemen. Given the high rates of co-resistance to β -lactams and aminoglycosides, empirical therapy for complicated UTIs should prioritize carbapenems (e.g., meropenem) and amikacin, especially in patients with healthcare-associated risk factors such as catheterization or recent hospitalization.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SAAA conceptualized the study, applied methodology, performed data collection and formal analysis. SSB performed validation. SSB and AYAJ supervised the study. AYAJ performed project administration. SAAA wrote the manuscript. SSB and AYAJ reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Ethics Committee, Faculty of Medicine and Health Sciences, Sana'a University, Republic of Yemen.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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