

# Molecular Characterizations and Antimicrobial Susceptibility of Extended-Spectrum ß-lactamase (ESBL) Producing Proteus spp. Clinical Isolates in Babol, **Northern Iran**

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Sciences, Qazvin, Iran ARTICLE INFO ABSTRACT Introduction: Proteus spp. are opportunistic members of Enterobacteriaceae. **Original Article** accounting for 10% of urinary tract infections and other primary clinical infections. They produce extended-spectrum beta-lactamases (ESBL) that can Keywords: Proteus spp, Betaconfer resistance to beta-lactam antibiotics. This study aimed to investigate the lactamase, Multi-drug resistance, prevalence, antimicrobial susceptibility, molecular characteristics, and genetic Antibiotic resistance relationship of ESBL-producing Proteus spp. clinical isolates in Babol, Northern Iran. Methods: In this cross-sectional study, out of 112 clinical Received: 10 Dec. 2021 samples, 30 Proteus spp. isolates were identified via specific biochemical Received in revised form: 21 Jun. assays. According to the Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines, antibiotic susceptibility was evaluated using disc diffusion and agar Accepted: 12 Jul. 2022 dilution methods, and polymerase chain reaction (PCR) was used to detect DOI: 10.52547/JoMMID.10.3.114 *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes. **Results:** The resistance rate to tetracycline and sulfamethoxazole was highest by disk diffusion and agar dilution. Multiple \*Correspondence drug-resistant (MDR) isolates were 86% and 60% in disk diffusion and agar Email: e.ferdosi@mubabol.ac.ir; elaheh.ferdosi@yahoo.com dilution assays. Seven (23.3%) isolates had the  $bla_{\text{TEM}}$  genes and 18 (60%) Tel: +989113210431 bla<sub>SHV</sub>. Conclusion: ESBL-producing Proteus spp. was highly prevalent, Fax: and the bla<sub>SHV</sub> was the most common resistance contributing gene. These © The Author(s) findings and relatively high resistance to ampicillin demand more care in prescribing antibiotics. Also, the high prevalence of MDR isolates in patients ☞◉ঙ≡

NC **INTRODUCTION** 

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*Proteus* spp. are opportunistic members of Enterobacteriaceae responsible for 10% of urinary tract infections, cystitis, polio-nephritis, prostatitis, ulcer, eye, and intra-abdominal infections. Proteus vulgaris, Proteus mirabilis, and Proteus penneri are common pathogens affecting immunosuppressed individuals. Also, Proteus members cause ~15% of nephrolithiasis through alkalinization. These bacteria were documented as extended-spectrum beta-lactamase (ESBL)-producers in 1987 [1]. Extended-spectrum  $\beta$ -lactamase (ESBL) producing Enterobacteriaceae is a public health concern worldwide [2, 3]. They produce enzymes responsible for the hydrolysis of oxyimino-beta-lactam antibiotics [4]. The spread of  $\beta$ -lactam antibiotic-resistant isolates occurs by a wide range of ESBL genes, e.g., blaTEM and  $bla_{SHV}$  [5, 6]. These narrow-spectrum  $\beta$ -lactamases are located on plasmid cassettes and contribute to resistance  $\beta$ -lactam antibiotics. The rapid increase in to cephalosporin-resistant Enterobacteriaceae containing *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes poses a major therapeutic challenge [6-8].

infected with ESBL-producing Proteus spp. requires continuous surveillance.

Misusing antibiotics has led to the spread of multi-drug resistant (MDR) strains, making it a significant challenge for the medical community. The ESBL resistance increases over time. In community-onset, there is a 0.91% yearly increase in ESBL, while in healthcare onsets, it reaches up to 2.31%. In some countries, the phenotypic ESBL production is estimated at 65% Enterobacteriaceae isolates, and in Europe, ESBL antibiotics were the first-line therapy for the associated

infectious diseases. Therefore, the first reports of resistance to ESBL antibiotics came from Europe, and it did not take long for similar reports to be received from around the world [9-13]. The gradual evolution of antibiotic-resistant strains has led to the expression of resistance genes in antibiotic-sensitive bacterial strains through gene mutation and horizontal gene transfer, increasing the MDRs prevalence worldwide [14, 15]. The class I integron is a common factor in distributing and spreading antimicrobial resistance. This class carries more than 40 resistance genes related to aminoglycosides, beta-lactams, chloramphenicol, macrolides, sulfonamides, and disinfectants [16].

This study investigated the *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> resistance genes in *Proteus* spp. and their correlations with antibiotic resistance patterns in hospitalized patients.

### MATERIAL AND METHODS

The setting, bacterial isolates, and study design. From March 2018 to April 2019, we collected 112 blood samples from the inpatients at Ayatollah Rohani Hospital, Babol, Northern Iran. *Proteus* spp. were identified based on conventional biochemical and microbiological tests, i.e., biotyping assays. All isolates were stored in Luria Bertani broth (Merck, Germany) containing 20% glycerol at -80 °C for further use.

The Ethical Committee of Babol University of Medical Sciences approved this study (Code No.: MUBABOL.REC.1394.162].

**Disk diffusion (DD) assay.** Susceptibility testing was performed with ten antibiotics by standard disk diffusion (DD) technique according to CLSI (Clinical and Laboratory Standards Institute) standard procedure [17]. The antibiotics included gentamicin (10  $\mu$ g), cefepime (30  $\mu$ g), amikacin (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), imipenem (10  $\mu$ g), cefotaxime (30  $\mu$ g), ampicillin (10 $\mu$ g),

piperacillin/tazobactam (30  $\mu$ g), sulfamethoxazole (100  $\mu$ g), and tetracycline (10  $\mu$ g) (MAST Diagnostics, Merseyside, UK). *Escherichia coli* ATCC 25922 was used as positive quality control.

Agar dilution (AD) method. After preparing stock solution from antibiotics according to CLSI 2018 standard [17],  $1.5 \times 10^8$  CFU/ml of microbial suspensions were cultured on Mueller-Hinton Agar containing the desired antibiotics (MAST Diagnostics, Merseyside, UK) and incubated at 37 °C for 18 to 24 h. A plate containing a medium with no antibiotics was included in assays as the negative control, and results were evaluated according to the CLSI2018 standard table.

Detection of blashy and blatem genes. According to the manufacturer's instructions, DNA extraction from all isolates was performed using a high pure PCR template preparation kit (Roche, Germany). The extracted DNAs were stored at -20 °C for subsequent steps. The ESBLencoding loci, *bla*<sub>TEM</sub>, and *bla*<sub>SHV</sub> were amplified by conventional PCR using the primers and conditions described by others (Table 1). The 60 µl PCR reactions contained 10 µl of extracted template DNA, 5 µl of 10x buffer, 1.5mM MgCl<sub>2</sub>, 0.2mM dNTPs, 50 pMole of each primer (Copenhagen, Denmark), 1.5U of Taq DNA polymerase (Amplicon Co., Denmark) and ddH<sub>2</sub>O to the final volume. Amplification was performed in a thermocycler (Corrbet, Australia) (Table 1), and PCR products were electrophoresed in 1.5% agarose gel. The PCR products were sequenced in both directions using the same primers used in amplification in an automated DNA sequencer device (Forster, USA). The standard strain integron-positive Proteus spp. (ATCC1209) was used as a positive control and integron-negative Proteus spp. (ATCC1053) as a negative control. The generated sequences were analyzed at the National Center for Biotechnology Information (NCBI), available at the (http://www.ncbi.nlm.nih.gov/BLAST/) website.

Table 1. Primers and PCR programs for amplifying *bla*TEM and *bla*SHV genes

Target	Primer	Primer sequence	Product size (bp)	No. Cycles	Denaturation	Annealing	Extension	Final Extension	Reference
bla <sub>тем</sub>	TEM-F	5'-ATGAGTATTCAACATTTCCG- 3'	851	30	94 °C for 30 S	55 °C for 30 S	72 °C for 1 min	72 °C for 4 min	[32]
	TEM-R	5'-TTAATCAGTGAGGCACCTAT- 3'	001						[92]
blasm	SHV-F	5'-ATGCGTTATATTCGCCTGTG- 3'	735	35	94 °C for 1 min	60 °C for 1	72°C for 1	72 °C for 10 min	[33]
Diashy	SHV-R	5'-TGCTTTGTTATTCGGGCCAA- 3'	155			min	min		

# RESULTS

**Bacterial Isolation.** From March 2018 to April 2019, 30 clinical *Proteus* spp. isolates were collected from 30 patients admitted to Ayatollah Rohani Hospital (Babol,

Northern Iran). Other isolates were excluded from the study.

PCR Condition

Antibiotic Resistance Profile. MDR was evaluated by DD assay and AD method. All strains were screened for resistance to 10 antimicrobials by DD. The resistance

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rates to tetracycline, sulfamethoxazole, ampicillin, cefotaxime, imipenem, gentamicin, cefepime and ciprofloxacin were 90%, 83.3%, 51.7%, 48.2%,17.2%, 13.7%, 10.3% and %3.4, respectively. In contrast, there was no resistance to amikacin and piperacillin/tazobactam. In the DD method, 86.6% of isolates were MDR phenotype. In the agar dilution

method, the resistance rate to sulfamethoxazole, ampicillin, tetracycline, cefotaxime, and cefepime were 80%, 50%, 13.7%, 13.7%, and 10.3%, respectively. No resistance to ciprofloxacin, gentamicin, amikacin, imipenem, and piperacillin/tazobactam was detected (Table 2). Also, in the AD method, 60% of isolates were MDR phenotype (Tables 3 and 4).

**Table 2.** Frequency of Antibiotic susceptibility pattern of *Proteus* spp. evaluated by disk diffusion (DD) and Agar dilution (AD) methods

Antibiotics	Method	Resistant	Intermediate	susceptible	Total number	<i>P</i> -value
Ciprofloxacin	Disk diffusion	<i>n</i> =1 (3.4%)	n = 2 (6.8%)	n = 27(90%)	<i>n</i> = 30	NS*
	Agar dilution	n = 0 (0.0%)	n = 1 (3.4%)	n = 29 (96.6%)	<i>n</i> = 30	
Amikacin	Disk diffusion	$n = 0 \; (0.0\%)$	n = 2 (6.8%)	n = 28 (93.3%)	n = 30	< 0.001
	Agar dilution	n = 0 (0.0%)	n = 0 (0.0%)	n = 30 (100%)	n = 30	
Tetracycline	Disk diffusion	n = 27 (90%)	$n = 0 \; (0.0\%)$	n = 3 (10.3%)	n = 30	< 0.001
	Agar dilution	n = 4 (13.7%)	n = 23 (76.6%)	<i>n</i> =3 (10.3%)	n = 30	
Gentamicin	Disk diffusion	n = 4 (13.7%)	$n = 0 \; (0.0\%)$	n = 26 (86.6%)	n = 30	NS
	Agar dilution	n = 0 (0.0%)	n = 3 (10.3%)	n = 27 (90%)	<i>n</i> = 30	
Cefotaxime	Disk diffusion	$n = 14 \; (48.2\%)$	n = 9 (31%)	n = 7 (23.3%)	n = 30	NS
	Agar dilution	n = 4 (13.7%)	n = 10 (34.4%)	n = 16 (53.3%)	n = 30	
Ampicillin	Disk diffusion	n = 15 (51.7%)	n = 3 (10.3%)	n = 12 (40%)	n = 30	NS
	Agar dilution	n = 15 (50%)	n = 0 (0.0%)	n = 15 (50%)	n = 30	
Cefepime	Disk diffusion	n = 3 (10.3%)	n = 21 (72.4%)	n = 6 (20%)	n = 30	< 0.05
-	Agar dilution	n = 3 (10.3%)	n = 0 (0.0%)	n = 27 (90%)	n = 30	
Imipenem	Disk diffusion	n = 5(17.2%)	n = 1 (3.4%)	$n = 24 \ (80\%)$	n = 30	NS
	Agar dilution	n = 0 (0.0%)	n = 5 (17.2%)	n = 25 (83.3%)	n = 30	
Piperacillin tazobactam	Disk diffusion	$n = 0 \; (0.0\%)$	$n = 0 \; (0.0\%)$	n = 30 (100%)	<i>n</i> = 30	< 0.001
	Agar dilution	n = 0 (0.0%)	n = 0 (0.0%)	n = 30 (100%)	n = 30	
sulfamethoxazole	Disk diffusion	$n = 24 \ (8.\%)$	$n = 0 \; (0.0\%)$	n = 6 (20%)	n = 30	< 0.001
	Agar dilution	$n = 24 \ (80\%)$	n = 0 (0.0%)	n = 6 (20%)	<i>n</i> = 30	

Table 3. Multi-drug resistant (MDR) pattern in isolated antibiotic-resistant Proteus spp.

Method	MDR	Antibiotics	Resistant sample count (%)	Total number (%)	
		T+ SXT	n = 5 (16.6%)		
	Double-resistant	T + CTX + SXT	n = 1 (3.3%)	n = 7 (23.3%)	
		T + CTX	n = 1 (3.3%)		
		T + GM + STX	n = 4 (13.3%)		
	Triple-resistant	T + CTX + SXT	n = 1 (3.3%)	0 (2001)	
uo		T + AP + CTX	n = 3 (10%)	n = 9 (30%)	(%
usi		T + CTX + CPM	n = 1 (3.3%)		6.6
diff	Quadruple-	T + CTX + AP + SXT	n = 4 (13.3%)		6 (8
isk	resistant	T + CTX + CPM + SXT	n = 2(6.6%)	n = 6 (20%)	- - -
D					u :
	Quintuplet- resistant	T + CTX + AP + CP + SXT	n = 3 (10%)	n = 3 (10%)	
	Sextuplet-	T + GM + CTX + AP + CP + SXT	n = 1 (3.3%)	n = 1 (3.3%)	
	resistant			n = 1 (5.570)	
	Double-resistant	AP + SXT	n = 10 (33.3%)		
		CTX + CPM	n = 2 (6.6%)	n = 12 (40%)	
ution		T + AP + SXT	<i>n</i> = 3 (10%)	5 (1 6 60()	
r dil	Triple-resistant	CTX + AP + SXT	n = 2 (6.6%)	n = 5 (16.6%)	(%0
∖gai	Quadruple-	T + CTX + CPM + SXT	n = 1 (3.3%)		9
Ą	resistant		· · ·	n = 1 (3.3%)	= 18

T = Tetracycline; CTX = Cefotaxime; SXT = sulfamethoxazole; GM = Gentamycin; Ap = Ampicillin; CPM = Cefepime; CP = Ciprofloxacin

PCR amplification of Gene Cassettes. Of 30 Proteus spp. isolates, 7 (23.3%) and 18 (60%) were positive for *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes, respectively (Fig. 1).

**BLAST** and Nucleotide Sequence Accession **Number.** The positive  $bla_{\text{TEM}}$  and  $bla_{\text{SHV}}$  strains were

sequenced and blasted against similar sequences in the Genbank database. After alignment, the homologous sequences were excluded, and the novel ones were deposited in the GenBank database under the accession numbers MH724198, MH724199, MH724200, and MH724201.



Fig. 1. PCR amplification of *bla*<sub>TEM</sub> (a) and *bla*<sub>SHV</sub> (b) genes. Lane M: DNA size marker (100bp); C-: negative control (ATCC 1053); C+: positive control (ATCC 1209).

Antibiotics	Antibiotic			Ant			
	evolution method	Presence/Abs	ence of Genes	Resistant	Sensitive	Total samples	P-value
	Agar Dilution	TEM	TEM+ TEM-	0 (0%) 1 (4.3%)	7 (100%) 22 (95.7%)	n = 7 $n = 23$	NS*
ıcin		SHV	SHV+	0 (0%)	18 (100%)	n = 18	NS
proflox	Disk Diffusion	TEM	SHV- TEM+ TEM-	1 (8.3%) 1 (14.3%) 2 (8.7%)	11 (91.7%) 6 (85.7%) 21 (91.3%)	n = 12 n = 7 n = 23	NS
Ū	Diffusion	SHV	SHV+ SHV-	1(5.6%) 2(16.7%)	17 (94.4%) 10 (83.3%)	n = 23 $n = 18$ $n = 12$	NS
, k A		TEM	TEM+	0 (0%)	7 (100%)	n = 7	< 0.001
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Table 4.	Correlation	hetween	blaTEM	and blaSHV	genes and	antibiotic	resistance
1 ant 7.	Conciation	Detween	UlaiLivi		genes and	annoione	resistance

							II.
	Agar		TEM-	0 (0%)	23 (100%)	<i>n</i> = 23	
	Dilution	SHV	SHV+	0 (0%)	18 (100%)	n = 18	< 0.001
			SHV-	0 (0%)	12 (100%)	n = 12	
	Disk	TEM	TEM+	0 (0%)	7 (100%)	n = 7	NS
	Diffusion	12111	TEM-	2(8.7%)	21 (91.3%)	n = 23	110
	Dinusion	SHV	SHV+	$\frac{1}{56\%}$	17(944%)	n = 18	NS
		5111	SHV-	1 (8 3%)	11 (91 7%)	n = 10 n = 12	115
			511 -	1 (0.570)	11 ()1.770)	n = 12	
	Agar	TEM	TEM+	6 (85.7%)	1 (14.3%)	n = 7	NS
	Dilution		TEM-	21 (91.3%)	2 (8.7%)	n = 23	
ne		SHV	SHV+	15 (83.3%)	3 (16.7%)	n = 18	NS
cli			SHV-	12 (100%)	0 (0%)	n = 12	
icy	Disk	TEM	TEM+	6 (85.7%)	1 (14.3%)	n = 7	NS
etre	Diffusion		TEM-	21 (91.3%)	2 (8.7%)	n = 23	
Ĕ		SHV	SHV+	15 (83.3%)	3 (16.7%)	n = 18	NS
			SHV-	12 (100%)	0(0%)	n = 12	
	<b>A</b> = = =	TEM	TEM	0 (00()	7 (1000()		NC
	Agar	IEM	IEM+	0(0%)	7 (100%)	n = 7	NS
	Dilution		TEM-	3 (13%)	20 (87%)	<i>n</i> = 23	
in		SHV	SHV+	1 (5.6%)	17 (94.4%)	n = 18	NS
nic			SHV-	2 (16.7%)	10 (83.3%)	n = 12	
itar	Disk	TEM	TEM+	0 (0%)	7 (100%)	n = 7	NS
jen	Diffusion		TEM-	4 (17.4%)	19 (82.6%)	n = 23	
0		SHV	SHV+	2(11.1%)	16 (88.9%)	n = 18	NS
			SHV-	2(16.7%)	10 (83.3%)	n = 12	
	Agar	TEM	TEM+	5(71.4%)	2 (28.6%)	n = 7	NS
	Dilution	12.01	TEM-	9 (39 1%)	14(60.9%)	n = 7 n = 23	115
	Dilution	SHV	SHV	10 (55.6%)	8(44.4%)	n = 23 n = 18	NS
ne		511 V	SHV	10(33.0%)	8 (66 7%)	n = 10 n = 12	115
ixi			511 V-	4 (33.370)	8 (00.770)	n = 12	
ots	Disk	TEM	TEM+	6 (85.7%)	1 (14.3%)	n = 7	NS
Gef	Diffusion		TEM-	17 (73.9%)	6 (26.1%)	<i>n</i> = 23	
0		SHV	SHV+	15 (83.3%)	3 (16.7%)	n = 18	NS
			SHV-	8 (66.7%)	4 (33.3%)	n = 12	
	Agar	TEM	$TEM_{\perp}$	4 (57 1%)	3 (12 9%)	n-7	NS
	Dilution	I LIVI	TEM	4(37.170) 11( $47.8\%$ )	12(52.2%)	n = 7 n = 23	115
	Dilution	SHV	SHV	9(50%)	0(50%)	n = 23 n = 18	NS
lin		511 V	SHV	9 (50%) 6 (50%)	9 (50%) 6 (50%)	n = 10 n = 12	115
cil			511 V-	0 (50%)	0 (50%)	n = 12	
iqn	Disk	TEM	TEM+	5 (71.4%)	2 (28.6%)	n = 7	NS
An	Diffusion		TEM-	13 (56.5%)	10 (43.5%)	n = 23	
		SHV	SHV+	10 (55.6%)	8 (44.4%)	n = 18	NS
			SHV-	8 (66.7%)	4 (33.3%)	n = 12	
	Agar	TEM	TEM+	2 (28.6%)	5 (71.4%)	n = 7	NS
	Dilution		TEM-	1 (4.3%)	22 (95.7%)	n = 23	
		SHV	SHV+	3 (16.7%)	15 (83.3%)	n = 18	NS
me		5111	SHV-	0 (0%)	12 (100%)	n = 12	115
ijdž	Disk	TEM	TEM+	5(714%)	2(28.6%)	n = 12 n = 7	NS
efe	Diffusion	12111	TEM-	19 (82 6%)	4(174%)	n = 23	115
0	Dinusion	SHV	SHV+	14(77.8%)	4 (22.2%)	n = 18	NS
		5111	SHV-	10(83.3%)	2(16.7%)	n = 10 n = 12	145
			511 (	10 (05.570)	2 (10.770)	n = 12	2.0
	Agar	TEM	TEM+	1 (14.3%)	6 (85.7%)	n = 7	NS
	Dilution		TEM-	4 (17.4%)	19 (82.6%)	n = 23	
-		SHV	SHV+	4 (22.2%)	14 (77.8%)	n = 18	NS
len			SHV-	1 (8.3%)	11 (91.7%)	n = 12	
рег	Dick	TEM	TEM	1(14.304)	6 (85 704)	n - 7	NS
Di	DISK	I LIVI		1(14.5%)	0(0.770)	n - 7	185
I	Diffusion		IEM-	5 (21.7%)	18 (78.5%)	n = 23	2.0
		SHV	SHV+	5 (27.8%)	13 (72.2%)	n = 18	NS
		-	SHV-	1 (8.3%)	11 (91.7%)	n = 12	
E	Agar	TEM	TEM+	0 (0%)	7 (100%)	n = 7	< 0.001
otai	Dilution		TEM-	0 (0%)	23 (100%)	n = 23	
baı		SHV	SHV+	0 (0%)	18 (100%)	n = 18	< 0.001
1ZO			SHV-	0 (0%)	12 (100%)	n = 12	
n tê	Disk	TEM	TEM+	0 (0%)	7 (100%)	n = 7	< 0.001
illi	Diffusion		TEM-	0 (0%)	23 (100%)	n = 23	
aci		SHV	SHV+	0 (0%)	18 (100%)	n = 18	< 0.001
рег			SHV-	0 (0%)	12 (100%)	n = 12	
Pij			5117	5 (575)	(100/0)	– 14	

e a u -	Agar	TEM	TEM+	5 (71.4%)	2 (28.6%)	n = 7	NS
zol xo zol	Dilution		TEM-	19 (82.6%)	4 (17.4%)	n = 23	
		SHV	SHV+	14 (77.8%)	4 (22.2%)	n = 18	NS
			SHV-	10 (83.3%)	2 (16.7%)	n = 12	
	Disk	TEM	TEM+	5 (71.4%)	2 (28.6%)	n = 7	NS
	Diffusion		TEM-	19 (82.6%)	4 (17.4%)	<i>n</i> = 23	
		SHV	SHV+	14 (77.8%)	4 (22.2%)	n = 18	NS
			SHV-	10 (83.3%)	2 (16.7%)	n = 12	

### DISCUSSION

The prevalence of ESBL- producing Proteus spp. is increasing worldwide, including in the United States, Asia, and Europe [18]. In the present study, the prevalence of  $bla_{\text{TEM}}$  and  $bla_{\text{SHV}}$  were 23.3% and 60%. These differences in the distribution of *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> may be due to geographical distribution, type of organisms, and source of infections. In Iraq, the prevalence of blaTEM was 60% among P. mirabilis isolates [19], while in China, it was around 52% in the same bacteria [20]. In Argentina, investigating the resistance to  $\beta$ -lactam/ $\beta$ -lactamase inhibitors in enterobacteria revealed that all Proteus spp. isolates harbored the  $bla_{\text{TEM}}$  gene [21]. In India, the  $bla_{\text{TEM}}$ rate among P. mirabilis isolates was 81.9% [22], while in Egypt, it was 35% [23]. In Tehran, Iran, Malekjamshidi et al. (2010) estimated bla<sub>TEM</sub> prevalence at 83% among ESBL-positive P. mirabilis specimens [24]. Other studies indicated variable rates ranging from 8.3% to 91% [25-29].

In a study by Hamid *et al.*, no *P. mirabilis* isolates in Iraq had  $bla_{SHV}$  [19]. In India, the  $bla_{SHV}$  prevalence among *P. mirabilis* isolates was 7% [22]. In Tehran, Iran, the prevalence of  $bla_{SHV}$  prevalence was 8% in ESBL-positive *P. mirabilis* isolates [24]

The correlation between  $bla_{\text{TEM}}$  and  $bla_{\text{SHV}}$  gene and resistance to some antibiotics showed a significant correlation. The *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes significantly correlated with the resistance to piperacillin/tazobactam obtained by the disk diffusion method. Also, there was a significant correlation between  $bla_{\text{TEM}}$  and  $bla_{\text{SHV}}$  genes and resistance to piperacillin/tazobactam and amikacin in the agar dilution method. According to the disk diffusion and agar dilution assays, piperacillin/tazobactam, amikacin, gentamicin, and imipenem are proper choices for treating *Proteus* spp. Given that most ESBL-positive strains showed increased resistance to tetracycline, sulfamethoxazole, cefotaxime, and ampicillin, blaTEM and blashy genes might help confer resistance to these antibiotics. Conza et al. (2014) showed a significant association between the  $bla_{\text{TEM}}$  gene and resistance to amoxicillin-clavulanic acid [21]. Also, Li et al. (2022) showed a substantial correlation between blaTEM and resistance to chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole in P. mirabilis isolates [20].

In our study, the highest antibiotic resistance rates were against sulfamethoxazole, tetracycline, and ampicillin. The results of MDR strains in both disk diffusion and agar dilution methods were 86% and 60%, almost similar to other studies in different countries [17, 26, 27, 30, 31]. Due to the high prevalence of MDR strains, which indicates misuse of antibiotics, studying the physiological properties of  $\beta$ -lactamase genes has received much attention. *Proteus* spp., an opportunistic bacterium, accounts for 10% of urinary tract infections. Therefore, identifying resistance genes is essential for implementing infection control programs and preventing the spread of resistant strains [10-13].

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#### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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