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Factors Related to Fecal Carriage of ESBL Producing *Escherichia coli* in Drug Users in Southwestern Iran

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ABSTRACT

Background: The escalating prevalence of multidrug-resistant (MDR) commensal intestinal bacteria characterized by extended-spectrum β-lactamase (ESBL) production is an alarming global health threat. Drug users have been introduced as a major source of antibiotic-resistant bacteria, possibly due to drug abuse. The present study aimed to investigate the potential factors related to fecal carriage of MDR ESBL-producing intestinal *Escherichia coli* (*E. coli*) in drug users in the southwest of Iran. .

Materials & Methods: In this cross-sectional study, stool samples of 109 drug users were collected and cultured. After the biochemical confirmation of *E. coli* isolates, the antimicrobial resistance pattern and ESBL production of the isolates were determined. Then logistic regression analysis was conducted to determine possible factors related to fecal carriage of MDR ESBL-producing intestinal *E. coli*.

Findings: Logistic regression analysis indicated that increasing age and duration of addiction were associated with increased risk of MDR ESBL-producing *E. coli* carriage in the intestinal flora of drug users ($p< .05$). Moreover, oral drug use compared to the smoking method led to a higher carriage rate of MDR ESBL-producing *E. coli* in the intestinal flora of drug users ($p< .05$). Also, self-employed drug users compared to those with fixed public occupation showed higher rates of MDR ESBL-producing *E. coli* carriage in their intestinal flora ($p< .05$).

Conclusion: Age, duration of addiction, method of drug use, and occupation were significantly associated with MDR ESBL-producing *E. coli* colonization.

Keywords: Drug users, Intestinal *Escherichia coli*, MDR, ESBLs, Risk factors, Logistic regression analysis.

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Introduction

Antibiotic resistance genes are frequently found on plasmids [1]. The presence of these genes in bacteria enables them to counteract with various antibiotics and render them ineffective [2]. These genes are rapidly evolving in terms of both abundance and quality, and nowadays, the presence of resistance genes poses a major challenge in the treatment of inpatients and outpatients [3]. Evidence suggests that the plasmid location of extended spectrum beta-lactamases (ESBLs) is correlated with multidrug resistance [4, 5]. The coexistence of resistance genes against different antibiotics, such as cephalosporins, aminoglycosides, tetracycline, quinolones, and carbapenems, on the same plasmid has been reported in the literature, and the co-presence of ESBL-encoding genes is assumed to be an advantage in the selection of concurrent processes [6–8]. Besides, the capacity of bacteria to acquire several plasmids leads to multidrug resistance in ESBL-producing strains, further limiting therapeutic alternatives against these pathogens [9]. Additionally, mobile genetic elements, such as insertion sequences, integrons, and transposons, play a significant role in facilitating the translocation of *bla* genes onto various plasmids, allowing the distribution of ESBLs among a broad range of hosts [10].

The *Enterobacteriaceae* family has attained various resistance genes in the past few years and is now mainly resistant to third-generation cephalosporin antibiotics as they harbor ESBLs. Today, this resistance mechanism is more common in *Escherichia coli* (*E. coli*), a microorganism that resides normally in the human gut [11]. One of the main risk factors for infection with antibiotic-resistant bacteria is the fecal carriage of multidrug resistant (MDR) and ESBL-producing *Enterobacteriaceae* species [12]. Fecal carriage creates favorable conditions for the horizontal transfer of antibiotic resistance genes among bacterial species [10]. Drug users, as the main transmitters of infection in communities, are probably the key contributors to endemic resistance in communities [13]. Addiction is generally considered a kind of social deviation and a social problem at higher levels. Some experts, emphasizing the individuality of addiction problems, believe that addiction is a victimless crime that does not directly harm others and should not be illegal as long as it does not violate the freedom of others [14]. Generally, the increasing occurrence of infectious diseases is seriously associated with drug use. Drug addicts could be carriers and spreaders of bacteria that could cause contagious diseases in their family members and others in the community. In other words, they could contribute to the proliferation and transmission of pathogens in the community [5, 14]. According to previous studies, gut colonization is a risk factor for infections with ESBL-producing bacteria [11, 15]. A previous study indicated that drug users played a vital role in spreading MDR ESBL-producing *E. coli* compared to non-addicted individuals [5]. However, there is a lack of comprehensive understanding regarding the factors influencing fecal carriage of MDR ESBL-producing *E. coli* in drug users.

Objectives: The present study aimed to identify risk factors associated with fecal carriage of MDR ESBL-producing intestinal *E. coli* among drug users.

Materials and Methods

Study design and sampling: This cross-sectional study was conducted on clients to a government toxicology laboratory in Ahvaz, Iran, from January 2019 to January 2020. After performing drug addiction screening tests and applying exclusion and inclusion criteria, 109 drug users provided their informed consent to participate in

the current study. Eligible participants who were addicted to one or more of the target addictive substances (opium, amphetamine, and or methamphetamine) had no active infection or antibiotic treatment in the six months prior to sample collection. At admission, participants completed a questionnaire concerning antibiotic use and infection history as well as name, age, gender, job, previous medical history related to antibiotic use, etc. Furthermore, blood samples were taken to test for hepatitis B and C infections. To isolate intestinal *E. coli* strains, stool samples were obtained from volunteers and cultured, and *E. coli* isolates were biochemically confirmed [5].

Possible risk factor variable data set: To recognize risk factors related to fecal carriage of MDR ESBL-producing *E. coli*, detailed information on a set of seven variables was collected from the study population, including age group, educational group, occupational group, type of drug used, drug use method, duration of addiction, and infection with hepatitis B or C.

Statistical analysis: For data analysis, SPSS (statistical package for social sciences) software Version 25.0 (IBM Corporation, Armonk, NY) was applied, univariate logistic regression analysis was used to express the results and determine the association between demographic factors (age group, educational group, occupational group, type of drug used, drug use method, duration of addiction, and infection with hepatitis B or C) and fecal carriage of MDR ESBL-producing *E. coli* in drug users. Besides, univariate and multivariate logistic regression analyses were used to describe possible associations between the presence of studied ESBL genes and multidrug resistance in intestinal *E. coli* isolates. To do this, univariate logistic regression analysis was first performed, and variables with a significance level of less than 0.1 in this analysis were included in the multivariate regression analysis model. Finally, variables with a significance level of less than 0.05 in the multivariate regression analysis were considered effective variables. The results of univariate and multivariate analyses were presented using odds ratio and adjusted odds ratio along with a 95% confidence interval.

Findings

Risk factors associated with fecal carriage of MDR ESBL-producing *E. coli*: Table 1 shows the influence of drug users' demographic factors on fecal carriage of genotypically, phenotypically, and both genotypically and phenotypically ESBL-positive MDR intestinal *E. coli* based on logistic regression analysis. Based on the statistical analysis results, age and duration of addiction were directly associated with fecal carriage of genotypically ESBL-positive isolates (p= .04). The prevalence of genotypically ESBL-positive isolates was significantly higher among drug users above 50 years of age compared to those aged 18-34 years old (p= .04). Additionally, individuals with a duration of addiction between 5-10 years and more than ten years had a higher incidence of genotypically ESBL-positive intestinal *E. coli* (p= .00) than those with an addiction of 3-5 years (p= .00).

Regarding fecal carriage of both genotypically and phenotypically ESBL-positive *E. coli*, the variables that showed a positive association with fecal carriage of ESBL-producing *E. coli* included age and method of drug use. The number of drug users above 50 years of age, who carried both genotypically and phenotypically ESBL-positive *E. coli* was notably higher than the number of those aged 18-34 years (p= .02). Among different methods of drug use, drug users who applied oral and smoking methods showed a positive association with fecal carriage of both genotypically and phenotypically

ESBL-positive *E. coli*, but the association was significant only for the oral way of drug use (p= .00).

The results also showed that fecal carriage of MDR isolates was significantly associated with age, occupational status, and method of drug use. The prevalence of MDR *E. coli* in drug users above 50 years of age was considerably higher than in those aged 18-34 years (p= .00); also, the prevalence was significantly higher among self-employed drug users (p= .04) compared to those with fixed public occupation. Users who used the oral method of drug use had a higher MDR *E. coli* carriage rate than those applying the smoking method (p= .02).

Association of ESBL genes with the phenotype of antibiotic-resistant isolates

Univariate logistic regression analysis: Univariate logistic regression analysis (Table 2) revealed that the presence of at least one ESBL gene had a significant and positive effect (p< .005) on the resistance of the isolates to the tested antibiotics, except for resistance to norfloxacin, chloramphenicol, and imipenem. In addition, the individual and simultaneous presence of ESBL genes had a significant direct effect on the multidrug resistance property of the isolates (p< .005).

Multivariate logistic regression analysis: Following univariate regression analysis (Table 2), multivariate logistic regression analysis showed that the presence of ESBL genes was not significantly associated with resistance to norfloxacin, penicillin, ceftriaxone, amoxicillin, imipenem, chloramphenicol, gentamycin, ciprofloxacin, tetracycline, and amikacin antibiotics. However, a direct and significant association was found between the presence of *bla*-CTX and *bla*-SHV genes and resistance to cefalotin (OR= 5.98, p= .01) and ceftazidime (OR= 13.94, p= .02) antibiotics, respectively. In addition, there was a positive relationship between the presence of the *bla*-TEM gene and resistance to antibiotics ampicillin (OR= 5.03, p= .02), trimethoprim-sulfamethoxazole (OR= 4.07, p= .02), and cefotaxime (OR= 14.81, p= .00). The results of multivariate regression analysis showed no significant association between the individual and simultaneous presence of the studied ESBL genes and multidrug resistance phenotype**s** of the isolates (Table3).

Discussion

Irrational use of antibiotics has been reported to be higher among drug users than in the general populations, and various studies have reported dangerous and inappropriate antibiotic usage patterns among drug users [14, 16, 17]. Self-medication with antibiotics without physician's supervision and prescription is one of the most harmful patterns [14]. Antibiotic abuse is a cause of drug resistance because overuse or misuse of antibiotics could lead to the acquisition of resistance genes in the normal microflora, which could then be transferred to pathogens. Thus, drug users are a potential reservoir of antibiotic resistance genes in the community [18].

A previous research showed that the prevalence of antibiotic-resistant intestinal *E. coli* was significantly higher among the drug-addicted population compared to the control population, with all fecal samples exhibiting resistance to at least two of the tested antibiotics [5]. The highest relative frequency of antibiotic resistance (94.5%) was related to erythromycin and penicillin, followed by amoxicillin and tetracycline with a relative frequency of 67 and 45%, respectively. Moreover, compared with the control population, fecal carriage of ESBL producing *E. coli* was more common among the addicted population, with the majority of ESBL strains (54.43%) being associated with MDR phenotypes (resistance to three or more antibiotics). The occurrence and

Table 1) Results of logistic regression analysis for the association between participants' demographic information and ESBL-producing *E. coli* carriage in drug users

Factors	No. (%) of Population		No. (%) of Samples with ESBL Positive E. coli												MDR (Isolates Resistant to ≥3 Antibiotics)			
			Phenotypically ESBL Positive *E. coli* (n)(%	Phenotypically ESBL Negative *E. coli* (n)(%)	OR (95% CI)	*P* Value	Genotypically ESBL Positive *E. coli* (n)(%)	Genotypically ESBL Negative *E. coli* (n)(%)	OR (95% CI)	*P* Value	*Phenotypically and Genotypically ESBL Positive *E. coli* (n)(%)	Phenotypically and Genotypically ESBL Negative *E. coli* (n)(%)	OR (95% CI)	*P* Value	No. (%) of Samples with MDR *E. coli* (n)(%)	No. (%) of Samples with no MDR *E. coli* (n)(%)	OR (95% CI)	*P* Value
Age group of drug users (n=109)	18-34 years old	24 (22)	20 (83.33)	4 (16.66)			16 (66.66)	8 (33.33)			15 (62.5)	9 (37.5)			10 (41.66)	14 (58.33)		
	35-50 years old	75 (68.8)	49 (65.33)	26 (34.66)	037 (0.11-1.21)	.09	45 (60)	30 (40)	0.75 (0.28-1.97)	.56	32 (42.66)	43 (57.33)	0.44 (0.17-1.14)	.09	23 (30.66)	52 (69.33)	0.61 (0.23-1.59)	.32
	Over 50	10 (9.2)	10 (100)	0	0 (0-0.37)	.17	10 (100)	0	0 (0-1.09)	.03	10 (100)	0	0 (0-1.32)	.02	10 (100)	0	0 (0-3.07)	.00
Educational group (n=109)	Diploma or lower	84 (77)	61 (72.61)	13 (15.47)			51 (60.71)	33 (39.28)			42 (50)	42 (50)			31 (36.90)	53 (63.09)		
	Associate's degree or BS	21 (19.3)	15 (71.42)	6 (28.57)	0.53 (0.171.63-)	.26	17 (80.95)	4 (19.04)	2.75 (0.858.89-)	.08	13 (61.90)	8 (38.09)	1.62 (0.61-4.32)	.33	10 (47.61)	11 (52.38)	1.55 (0.59-4.07)	.37
	MS and higher	4 (3.7)	3 (75)	1 (15)	0.639 (0.066.644-)	.70	3 (75)	1 (25)	1.94 (0.1919.46-)	.57	2 (50)	2 (50)	1 (0.13-7.43)	.00	2 (50)	2 (50)	1.70 (0.22-12.75)	.60
Occupational group (n=109)	Fixed public or quasi-public occupation	37 (34)	19 (51.35)	18 (48.64)			25 (67.56)	12 (32.43)			15 (40.54)	22 (59.45)			13 (35.13)	24 (64.86)		
	Self-employment	72 (66)	60 (83.33)	12 (16.66)	0.61 (0.321.18-)	.14	46 (63.88)	26 (36.11)	0.84 (0.361.96-)	.70	42 (58.33)	30 (41.66)	2.05 (0.91-4.59)	.08	40 (55.55)	32 (44.44)	2.30 (1.01-5.23)	.04
Type of drug used (n=109)	Opium and related substances	87 (80)	59 (67.81)	18 (20.68)			53 (60.91)	34 (39.08)			45 (51.72)	42 (48.27)			39 (44.82)	48 (55.17)		
	Amphetamine	14 (13)	12 (85.71)	2 (14.28)	0.79 (0.341.83-)	.58	11 (78.57)	3 (21.42)	2.35 (0.619.04-)	.20	7 (50)	7 (50)	0.93 (0.30-2.88)	.90	4 (28.57)	10 (71.42)	0.49 (0.14-1.69)	.25
	Methamphetamine	8 (7)	7 (87.5)	1 (12.5)	4.74 (0.5639.59-)	.11	7 (87.5)	1 (12.5)	4.49 (0.52-38.13)	.13	5 (62.5)	3 (37.5)	1.55 (0.34-6.91)	.56	2 (25)	6 (75)	0.41 (0.07-2.14)	.28
Method of drug usage (n=109)	Oral	39 (36)	30 (76.92)	9 (23.07)			28 (71.79)	11 (28.20)			27 (69.23)	12 (30.76)			21 (53.84)	18 (46.15)		
	Smoking	61 (56)	40 (65.57)	21 (34.42)	0.57 (0.221.42-)	.22	38 (62.29)	23 (37.70)	0.64 (0.271.54-)	.33	25 (40.98)	36 (59.01)	0.3 (0.13-0.72)	.00	19 (31.14)	42 (68.85)	0.38 (0.16-0.88)	.02
	Injection	9 (8)	9 (100)	0	-	.11	5 (55.55)	4 (44.44)	0.49 (0.112.17-)	.34	5 (55.55)	4 (44.44)	0.55 (0.12-2.44)	.43	3 (33.33)	6 (66.66)	0.42 (0.09-1.96)	.27
Duration of addiction (n=109)	Below 3 years	21 (19.3)	15 (71.42)	6 (28.57)			10 (47.61)	11 (52.38)			8 (38.09)	13 (61.90)			3 (14.28)	18 (85.71)		
	3-5 years	59 (54)	40 (67.79)	19 (32.20)	0.84 (0.28-2.51)	.76	34 (57.62)	25 (42.37)	1.49 (0.55-4.06)	.43	30 (50.84)	29 (49.15)	1.68 (0.60-4.65)	.31	21 (35.59)	38 (64.40)	3.31 (0.87-12.58)	.07
	5-10 years	21 (19.3)	18 (85.71)	3 (14.28)	2.40 (0.51-11.26)	.26	19 (90.47)	2 (9.52)	10.45 (1.92-56.63)	.00	13(61.90)	8 (38.09)	2.64 (0.75-9.17)	.12	13 (61.90)	8 (38.09)	9.75 (2.16-43.98)	.00
	Over 10 years	8 (7.4)	6 (75)	2 (15)	0.53 (0.12-2.20)	.38	8 (100)	0	-	.00	6 (75)	2 (25)	4.87 (0.78-30.28)	.07	6 (25)	2 (25)	18 (2.40-134.83)	.00
Infection with hepatitis (n=83)	Infected with hepatitis B	14 (16.86)	10 (71.42)	4 (28.57)			9 (64.28)	5 (35.71)			8 (57.14)	6 (42.85)			8 (100)	0		
	Infected with hepatitis C	69 (83.13)	55 (79.71)	14 (20.28)	1.57 (0.425.76-)	.50	52 (75.36)	16 (23.18)	1.80 (0.526.16-)	.34	40 (57.97)	29 (42.02)	1.03 (0.32-3.30)	.95	35 (50.72)	34 (49.27)	-	.00

* indicates the isolates harboring at least one of the target ESBL genes in addition to being phenotypically ESBL producer.

Table 2) Results of univariate logistic regression analysis for the association between ESBL genes and antibiotic resistance and multidrug resistance in intestinal *E. coli* isolates

	ESBL Genes	Odds Ratio	Conf-Interval 95%	*P* Value	ADJ Odds Ratio	Conf-Interval 95%	*P* Value
NOR	*bla*-CTX	3.28	0.35-30.42	.29	7.02	62.33-0.79	.08
	bla-SHV	2.70	0.29-25.1	.38	3.28	0.57-18.76	.18
	bla-TEM	-	-	-	-	-	-
CF	*bla*-CTX	8.49	2.35-30.600	.00	5.98	1.44-24.8	.01
	bla-SHV	4.87	1.54-15.38	.00	1.42	0.30-6.55	.65
	bla-TEM	-	-	-	-	-	-
P	*bla*-CTX	5.48	0.59-50.85	.13	-	-	-
	bla-SHV	8.55	0.96-75.96	.05	3.06	0.22-42.22	.40
	bla-TEM	10.60	1.19-94.44	.03	5.37	0.39-73.45	.21
CRO	*bla*-CTX	6.84	1.47-31.86	.01	3.23	0.60-17.10	.16
	bla-SHV	6.02	1.30-27.88	.02	1.80	0.32-10.1	.50
	bla-TEM	10.76	1.36-84.68	.02	4	0.41-38.58	.23
AMX	*bla*-CTX	2.65	1.15-6.08	.02	1.40	0.47-4.14	.53
	bla-SHV	2.95	1.28-6.78	.01	1.62	0.42-6.21	.48
	bla-TEM	3.43	1.47-8.003	.00	2.07	0.65-6.58	.21
CAZ	*bla*-CTX	2.55	0.84-7.70	.10	1.23	0.32-4.68	.75
	bla-SHV	7.11	1.55-32.60	.01	13.94	1.40-38.69	.02
	bla-TEM	-	-	-	-	-	
IPM	*bla*-CTX	1.40	0.55-3.57	.47	-	-	-
	bla-SHV	2.31	0.83-6.40	.11	-	-	-
	bla-TEM	1.81	0.65-5.03	.25	-	-	-
C	*bla*-CTX	4.18	0.47-37.08	.20	-	-	-
	bla-SHV	1.32	0.23-7.55	.75	-	-	-
	bla-TEM	1.07	0.187-6.15	.94	-	-	-
GM	*bla*-CTX	1.48	0.46-4.76	.51	-	-	-
	bla-SHV	4.55	0.96-21.48	.05	2.01	0.34-11.7	.43
	bla-TEM	8.29	1.04-66.07	.05	5.36	0.52-54.56	.16

NOR: norfloxacin, CF: cefalotin , P: penicillin, CRO: ceftriaxone, AMX: amoxicillin, CAZ: ceftazidim, IPM: imipenem, C: chloramphenicol, GM: gentamycin

co-occurrence rates of three common ESBL genotypes (*bla*-TEM, CTX-M, and SHV) were alarmingly high among *E. coli* strains isolated from drug users, emphasizing the significance of drug users as reservoirs of resistance genes in their normal flora. The combined results of phenotypic and genotypic analyses performed to detect ESBL producers showed that there may not be a direct correlation between a specific ESBL phenotype and the presence of a specific type of *bla* genes, specifically when all three *bla* genes are absent or present in the same isolate. Furthermore, isolates containing multiple types of *bla* genes demonstrated higher levels of phenotypic resistance compared to those harboring only one type of *bla* gene. More importantly, phenotypically non-ESBL producers were found to carry target ESBL genes, representing that this population should be considered as a hidden reservoir for the transfer of resistance genes

Table 3) Results of multivariate logistic regression analysis for the association between ESBL genes and antibiotic resistance and multidrug resistance in intestinal *E. coli* isolates (continued)

	ESBL Genes	Odds Ratio	Conf-Interval 95%	*P* Value	ADJ Odds Ratio	Conf-Interval 95%	*P* Value
AM	*bla*-CTX	1.52	0.68-3.39	.30	-	-	-
	bla-SHV	2.02	0.88-4.61	.10	0.50	0.13-1.83	.30
	bla-TEM	4.06	1.58-10.45	.00	5.03	1.79-16.94	.00
CP	*bla*-CTX	3.97	0.814-19.34	.09	2.76	0.51—14.88	.24
	bla-SHV	3.23	0.66-15.77	.15	-	-	-
	bla-TEM	-	-	-	-	-	-
SXT	*bla*-CTX	3.56	1.55-8.17	.00	1.35	0.48-3.78	.57
	bla-SHV	4.81	1.99-11.63	.00	1.04	0.28-3.75	.95
	bla-TEM	6.88	2.55-18.52	.00	4.07	1.19-13.83	.02
TET	*bla*-CTX	3.08	1.37-6.90	.00	0.74	0.07-7.01	.80
	bla-SHV	9	3.47-23.32	.00	3.49	0.14-85.47	.44
	bla-TEM	2.35	1.03-5.39	.04	-	-	-
CTX	*bla*-CTX	3.67	1.34-10.07	.01	1.22	0.368-4.09	.74
	bla-SHV	5.57	1.77-17.50	.00	0.67	0.13-3.23	.62
	bla-TEM	22.70	2.94-175.17	.00	14.81	163-134.22	.02
AN	*bla*-CTX	1.88	0.60-5.86	.27	-	-	-
	bla-SHV	3.27	0.87-12.25	.08	1.22	0.27-5.50	.80
	bla-TEM	9.91	1.255-78.25	.03	8.71	0.89-84.86	.06
MDR (resistance to≥3)	*bla*-CTX	12.30	2.63-57.50	.00	3.99	0.635-25.10	.14
	bla-SHV	17.14	3.67-80.02	.00	6.29	0.95-41.40	.06
	bla-TEM	8.71	2.59-29.24	.00	1.46	0.28-7.41	.65
MDR (resistance to≥3)	*bla*-CTX + *bla*-SHV	5.10	1.37 – 18.97	.01	-	-	-
	bla-CTX + *bla*-TEM	5.83	1.56 – 21.71	.00	-	-	-
	bla-SHV + *bla*-TEM	4.83	1.46 – 15.96	.01	-	-	-
	bla-CTX + *bla*-SHV + *bla*-TEM	3.32	0.89 – 12.83	.07	-	-	-

AM: ampicillin, CP: ciprofloxacin, SXT: trimethoprim-sulfamethoxazole, TET: tetracycline, CTX: cefotaxime, AN: amikacin

to pathogens. While the presence of *bla*SHV and *bla*TEM genes has been associated with ESBL phenotypes in some cases, this is not always the case, and additional analysis is needed to confirm that the isolates are indeed ESBL producers [5].

The current research focused on examining the potential risk factors associated with fecal carriage of MDR ESBL-producing commensal *E. coli* in the intestinal microflora of 109 drug users in Ahvaz, southwestern Iran. To the best of our knowledge, this is the first study conducted to examine the association between fecal carriage of MDR ESBL-producing intestinal *E. coli* and factors such as age, education, occupation, type of used drug, method and duration of drug use, and hepatitis B/C virus infection in a

population of drug users. There is a lack of information on carriage of MDR strains in drug users, but this population has risk factors related to carriage of MDR strains. Several studies have shown that addictive substances have an immunomodulatory effect on drug users, making them more vulnerable to infection [19]. Subsequently, following unreasonable patterns of antibiotic use increases the possibility of MDR carriage in the commensal microflora of drug users. Self-directed antibiotic therapy and poor adherence to prescribed antibiotic regimens are the most important patterns of irrational use of drugs (IUDs), leading to delays in appropriate antibiotic therapy initiation and completion, thereby contributing to drug resistance [14].

The current study could lay the foundation for identifying factors other than IUDs, contributing to the prevalence of MDR ESBL-producing *E. coli* in the intestinal microflora of the target population. Based on the univariate logistic regression analysis results, it was found that age above 50 years was directly associated with fecal carriage of genetically and phenotypically ESBL-positive MDR isolates. It was also found that 5-10 years and >10 years of addiction were positively associated with carriage of genotypically ESBL-positive isolates. Smoking and oral methods of drug consumption were associated with fecal carriage of phenotypically ESBL-positive and MDR intestinal *E. coli*, respectively. Besides, self-employment exhibited a strong association with fecal carriage of MDR isolates. In contrast, two related studies that assessed risk factors associated with ESBL-producing *E. coli* carriage in non-addicted community subjects found no strong association between the studied risk factors and fecal carriage of ESBL-producing *E. coli*; in addition, conflicting results have been reported in previous studies. This may be due to variations in study methodologies, sample populations, and geographic locations. The multifaceted nature of ESBL-producing *E. coli* carriage suggests that its dynamics are influenced by a complex interplay of factors that differ across diverse communities. Therefore, divergent findings in recent and past studies underscore the need for comprehensive investigations to discern specific factors contributing to ESBL-producing *E. coli* prevalence in different community settings [20, 21].

The presence of each ESBL gene in the isolates was strongly associated with antibiotic resistance, except for resistance to norfloxacin, chloramphenicol, and imipenem. The multivariate analysis results showed a strong association between *bla*-CTX and *bla*-SHV genes and resistance to cefalotin and ceftazidime antibiotics, respectively, with different odds ratios. The presence of the *bla*-TEM gene was associated with resistance to ampicillin and trimethoprim-sulfamethoxazole. Relevant studies have also reported a close relationship between ESBL production and ciprofloxacin resistance in *Klebsiella pneumoniae* and co-resistance to other antibiotic families, including fluoroquinolones, aminoglycosides, tetracycline, chloramphenicol, and sulfonamides [22–24].

This study also investigated potential associations between ESBL genes and MDR phenotypes in the studied isolates. The association between multidrug resistance phenotypes and presence or co-presence of *bla*-CTX, *bla*-SHV, and *bla*-TEM genes was confirmed by univariate analysis, but multivariate analysis showed no significant association. ESBL-producing strains commonly exhibit MDR phenotypes because MDR genes and plasmids are carried by the *Enterobacteriaceae* family. Therefore, the expression and coexistence of drug resistance genes make these

isolates potent resistance gene donors to other microorganisms [25–28].

Conclusion

Among drug users in Ahvaz (southwest of Iran), the variables of age, duration of addiction, method of drug use, and occupation were significantly associated with MDR ESBL-producing *E. coli* colonization. Understanding these factors could potentially improve public health interventions to prevent the spread of drug-resistant *E. coli* strains. However, further studies in other geographic locations are needed to generalize the results and establish public health interventions that could reduce the irrational use of drugs and subsequent colonization of drug-resistant bacteria in drug users.

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