Iran J Public Health, Vol. 51, No.12, Dec 2022, pp.2753-2763

Original Article

Evaluation of Antibiotic Resistance and *adeABC*, *adeR*, *adeS* Efflux Pump Genes among Foodborne and Clinical *Acinetobacter* spp. in Türkiye

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(Received 16 May 2022; accepted 14 Jul 2022)

Abstract

Background: The adeABC efflux pump has a crucial role in the resistance of *Acinetobacter baumannii* strains to antimicrobial agents; it is encoded by *adeABC, adeR, adeS* genes. We evaluated antibiotic resistance, efflux pump genes, clonal relationships, and analyzed a probable correlation that can exist between antibiotic resistance and the aforementioned genes.

Methods: We conducted this study on 27 food-originated and 50 human clinical *Acinetobacter* spp. in Southern Türkiye. MALDI-TOF system and disc diffusion/agar dilution (colistin) methods were used for the identification and antibiotic susceptibility. The efflux pump genes and genetic relatedness of the two groups were investigated by (PCR) and (PFGE) methods.

Results: Foodborne *A. dijkshoorniae* strain was multidrug- resistant (MDR), and none of them resistant to colistin. Most of the clinical isolates (92%) were Extensive-Drug Resistant (XDR); highest resistant to ceftazidime, piperacillin-tazobactam, and imipenem (47, 94%), and were lowest to colistin (7, 14%), respectively. *adeABC*, and *adeR, adeS* genes were (23, 85.2%), (9, 33.3%), (27, 100%) and (10, 37.3%), (18, 66.7%) in foodborne strains respectively. These rates were (43, 86%), (48, 96%), (50, 100%), and (34, 68%), (48, 96.7%) in clinical strains respectively. A positive correlation existed between *adeA* gene positivity and piperacillin-tazobactam, ceftazidime, gentamycin, imipenem (*P*=0.048), amikacin (P=0.007) and trimethoprim-sulfamethoxazole (*P*=0.029) resistance in clinical strains. A positive correlation of trimethoprim-sulfamethoxazole resistance and *adeS* gene positivity was seen in foodborne strains (*P*=0.018).

Conclusion: Multiple-efflux pump genes rise in parallel to multidrug-resistance in clinical isolates, while susceptible to diverse antibiotics; food may be a potential provenance for the dissemination of *adeABC*, *adeR* and *adeS* genes.

Keywords: *Acinetobacter* spp.; Efflux pump; Gene

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Introduction

Acinetobacter spp. are a Gram-negative genus, frequently found in the food industry. These bacteria can survive in numerous places; aquaculture or many outdoor environments (soil, water, and sewage) exhibits metabolic versatility, grows at low temperatures, and can form biofilms (1,2). In the last decades, *A. baumannii* has been the most common nosocomial infection agent, especially in intensive care units. Additionally, because of inappropriate/overdose antibiotic usage in agriculture, aquaculture, veterinary and medical areas, and the extension of *A. baumannii* Multi-Drug Resistant (MDR) strains, its morbidity and mortality have increased around the world (3).

It causes serious infections such as pneumonia, sepsis, urinary infections, wound infections, and even causes infections that result in death. Therefore, it has been considered one of ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), microorganisms by the WHO and it has been recommended to conduct epidemiological studies around the world (4,5). MDR in this species is mainly due to the horizontal acquisition of resistance genes. However, chromosomal gene expression for efflux systems, which play an important role in MDR, has increased in recent studies (5). The overexpression of the adeABC efflux pump is associated with carbapenem and cephalosporin resistance of *A. baumannii*. The adeABC is a three-component flow pump, a member of the Resistance-Nodulation-Split (RND) family. The AdeB component expels antibiotics from the cell, while adeA is a membrane fusion protein and AdeC is an outer membrane protein. The function of the *adeABC* flow pump is regulated by the *adeRS* two-component system. Point mutations in the *adeRS* operon can lead to increased expression of the efflux pump and thus to antibiotic resistance. Other efflux pumps; *adeIJK* and *abeM* have also been shown to contribute to imipenem and cephalosporin resistance (2,5).

It is crucial to regulate natural genes in adapting to changing environmental conditions and to obtain useful genes from the environment so the bacteria can survive. Similarly, *A. baumannii* has acquired several resistance determinants such as transposons, plasmids, genomic islands (GIs), insertion sequences (IS), and integrons from its environment by the way of horizontal gene transfer observed in its genome analyses (3,5). *Acinetobacter* spp. have also been reported in various foods and food-producing animals, which might constitute an unobserved source of bacterial pathogens to the human population. Although there are many studies on these resistance mechanisms/genes in clinical *A. baumannii* strains, there are fewer studies on whether these resistance mechanisms of the food chain are involved in the spreading of the resistance genes (4,5-7).

In this study, five efflux pump genes (*adeA*, *adeB*, *adeC*, *ade*R, and *adeS*) were evaluated in 50 *A. baumannii* strains of clinical origin and 27 food-borne *Acinetobacter* spp. In addition, it was statistically examined whether there was any correlation between *adeABC*, *ade*R, and *adeS* genes frequency and the antibiotic resistance in all strains. Moreover, the study at hand has checked whether the food and clinically derived *A. baumannii* strains were clonally related or not before undergoing genotypically comparisons with the PFGE method. To the best of our knowledge, this is the first study about this subject in our country.

Materials and Methods

Ethics approval

The Turkish Republic, University of Cukurova, Faculty of Medicine Ethical Board of Scientific Research' approved an etic report for this study with 89 decision code numbers on 14.06.2019.

Statistical analysis

The S-PLUS 20 statistics program (S-PLUS 20.00 for Windows, Professional Edition) was used. The chi-square test (Fisher's exact test and, where appropriate, Mann-Whitney U tests) was used to compare categorical measures (phenotypic and genotypic experiments) between the groups. A *P* value of <0.05 was considered statistically significant.

Bacterial strains and growth conditions

In the present study, 27 *Acinetobacter* spp. were isolated and identified from 102 food samples [n=39 fruit and vegetables, n=25 cheese, n=17 sucuk, n=13 chicken meat, and n=8 veal] purchased in 12 markets from 2019 Mar to Sep 2021. Clinical strains were collected over a period of 3 years (2019-2021) from clinical laboratories of the Department of Medical Microbiology, University of Çukurova (Türkiye). One isolate from each patient was collected from infected hospitalized patients (n=50).

Isolation and identification of foodborne and clinical *Acinetobacter* spp.

Isolation of foodborne isolates was evaluated, as described (5,6). Further species-level identification of isolates with a typical *Acinetobacter* spp. morphology on Tryptone Soy Agar (TSA) plates containing 5% sheep blood (Oxoid) media were fulfilled by biochemical tests; catalase production, Gram staining, motility, and oxidase tests. The presumptive colonies were identified using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF) (Bruker, Germany) (8). Clinical isolates were grown on Mac Concey and Columbia agar (Becton-Dickinson, Sparks, MD) supplemented with 5% defibrinated sheep blood (Oxoid). For identification, cultures were examined with MALDI-TOF automated identification system. The bla_{OXA-51} gene carriage was analyzed for confirmation of *A. baumannii* with the PCR method.

Detection of Oxa-51 and adeABC, adeR and adeS efflux pump genes

The boiling method was used to extract the genomic DNA previously described (9). In order to quantify the DNA samples (100ng/μL DNA for each sample), a spectrophotometer (UV-VIS Spectrophotometer CHEBIOS) was used.

Multiplex PCR was performed to screen specific *oxa-51*, *adeABC, ade*R, and *adeS* genes as described previously (10,11). Specific primers are presented in Table 1.

Table 1: List of primers used in the present study

Genes	*Primers sequence (5'-3')*	*Product size (bp)*	*Reference*
oxa-51	F:GACCGAGTATGTACCTGCTTCGACC R:GAGGCTGAACAACCCATCCAGTTAACC	497	(10)
adeA	F:GAAATCCGTCCGCAAGTC R:ACACGCACATACATACCC	683	(11)
adeB	F:AAAGACTTCAAAGAGCGG R:TCACGCATTGCTTCACCC	623	
adeC	F:ATTTCAGGTCGTAGCATT R:TTGATAAGTAGAGTAGGGATT	370	
*ade*R	F:AGATTTCATCTTTGATTCTTGG R:AATTGATTCTTAGCATCTGG	557	
adeS	F:ACTCGGGGATTGATAGGC R:GCTGCTAAAGCTGCGCTT	829	

The PCR mix to be used for one sample: In a total volume of 50 µL, PCR was performed. Each reaction contained 25 mM of MgCl2, 5 µL of 10X PCR buffer, 5 units/µL of Taq DNA polymerase (Fermentas-Lithuania), 1.25 mM of the dNTPs mix, 10 µM of reverse and forward primers, and 5 µL of template DNA. The remainder for each reaction was made up to 50 µL with water. The thermal profile involved 3 min at 94 °C (for *oxa-51*; 4 min) for initial denaturation step, 40 cycles of 30 sec at 94 °C (for *oxa-51*; 35 cycles), primer annealing temperature was set up for *oxa-51*, *adeA*, *adeB*, *adeC* and *adeS* at 55 °C for 30 sec, for *adeR* at 52 °C for 30 sec. The extension was also set up at 72 °C for 40 sec (for *oxa-51;* 1 min). The cycling was done according to a final extension step at 72 °C for 10 min (for *oxa-51;* 7 min). PCR products were analyzed by 2% agarose gel and stained with ethidium bromide.

Antibiotic susceptibility analysis

For this purpose, Kirby-Bauer disk diffusion method using 9 discs and including ciprofloxacin (CIP, 5 µg), piperacillin-tazobactam (TZP 100/10 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM, 10 µg), meropenem (MEM, 10 µg), amikacin (AK, 30 µg), trim./süfometaksazol (10 µg), gentamicin (10 Mg), and tetracycline (30 Mg), (all discs from Oxoid Ltd.). Susceptibility or resistance was determined according to the recommendation of (CLSI) guidelines (12). An index strain of *A. baumannii* (CLI-74) that nosocomial infection agent was used as a reference strain in antibiotic resistance and genotyping tests obtained from the Medical Microbiology Laboratory. Colistin (CL) susceptibility was determined by the broth microdilution method according to EUCAST guidelines (13,14).

Minimal inhibitory concentration (MIC) testing

The MICs of tested antibiotics were determined with the disk diffusion method (Biodisk, Solana, Sweden) according to the CLSI's(12) guidelines. For colistin, MIC was determined by the broth microdilution protocol according to the CLSI and EUCAST. Escherichia coli ATCC 25922 was used as a quality control strain. Isolates with a MIC ≤ of 2 µg/L for colistin were considered susceptible (13,14).

Pulsed-field Gel electrophoresis (*PFGE) analysis*

A macrorestriction analysis of chromosomal DNA with *ApaI* (New England Biolabs, Boston, Mass.) was evaluated with the PFGE method previously described (15).

Results

Overall, 27 *Acinetobacter* spp. were identified from 102 food samples (Table 2). Fifty clinical isolates were identified *A. baumannii*. These strains were isolated from aspiration fluid samples (n=14, 28%), wound (n=11, 22%), blood (n=8, 16%), urine (n=7, 14%), and other samples (n=10, 20%) respectively.

Antibiotic susceptibilities and efflux pump genes among the *Acinetobacter* spp. and their sources are presented in Table 2 and Fig. 1. PCR products of *adeABC*, *adeR,* and *adeS* genes are shown in Fig. 2. The correlations between efflux pump genes and antibiotic resistance patterns of the strains is presented in Tables 3 and 4. PFGE analyses; there were no clonal relations between foodborne and clinical strains. Both groups have separate related or unique clusters (Data not shown).

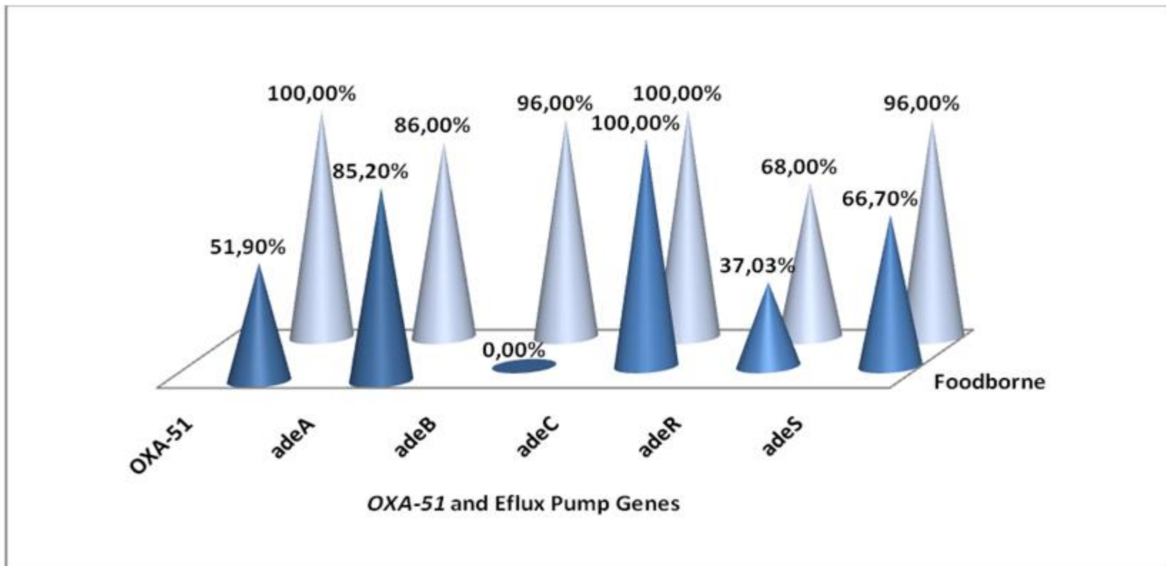

Fig. 1: Distribution of *oxa-51*, *adeABC, adeR,* and *adeS* efflux pump genes of *Acinetobacter* spp. from foodborne and clinical samples

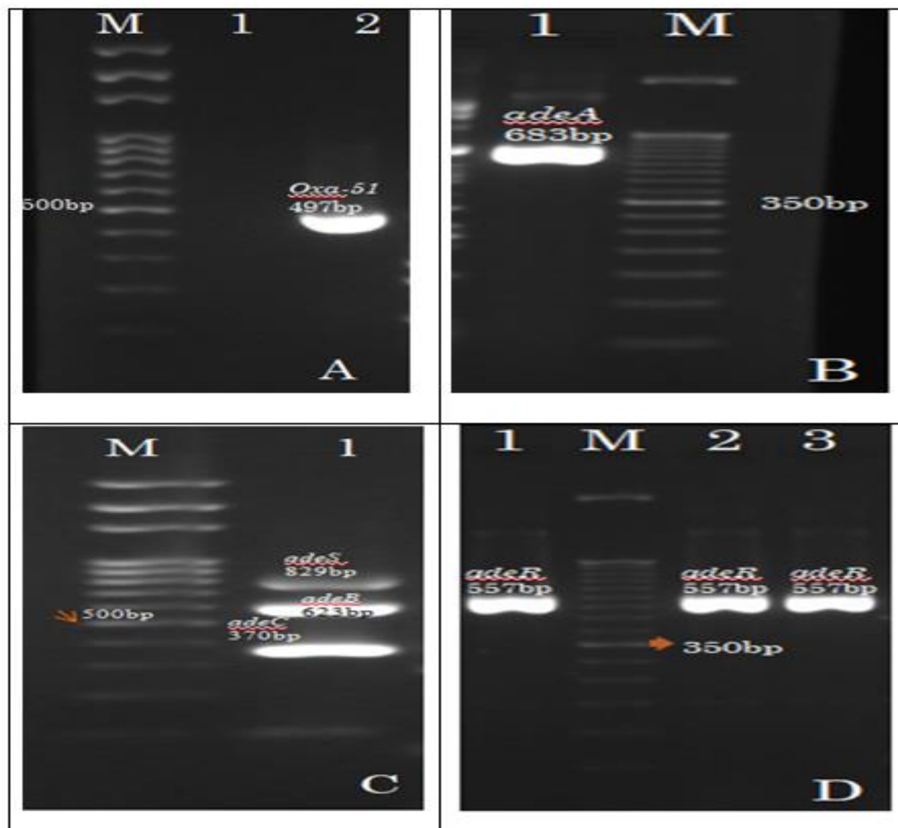

Fig. 2: PCR results of *oxa-51, adeABC*, *adeR, adeS* efflux pump genes. (A): (*Oxa-51)*; M: Marker (100bp), 1: negative control, 2: *A. pittii* (cheese): (B): (*adeA);* 1: *A. baumannii* (veal), M: Marker, (C): (*adeS, adeB, adeC*)*;* M: Marker (100bp), 1: *A. pittii* (cheese). (D): *(adeR*); 1: *A. bauannii* (lettuce), M: Marker (50bp), 2: *A. baumannii* (veal), 3: *A. baumannii* (sucuk)

Table 2: Antibiotic resistance profile among *Acinetobacter* spp. (*n*=77)

Species No.(%)	SXT			CIP			TZP			TE			AK		
	R	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*
A. Baumannii(50)	44(88)	3(6)	3(6)	45(90)	2(4)	3(6)	47(94)		3(6)				46(92)	1(2)	3(6)
Total(50)	44(88)	3(6)	3(6)	45(90)	2(4)	3(6)	47(94)		3(6)				46(92)	1(2)	3(6)
A. Baumannii(14)		2(14.3)								2(14.3)					
A. Baylyi(1)															
A. bereziniae (2)															
A. Calcoaceticus(1)															
A. dijkshoorniae (2)	1(50)									1(50)					
A. pittii(5)	1(20)														
A. schindleri (1)															
A.tandoi(1)															
Total(27)	2(7.4)	2(7.4)								3(11.1)					

Table 2: Continued…..

Species No.(%)	*MEM*			*CL*			*CAZ*			*CN*			*IMP*		
	R	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*	*R*	*I*	*S*
A. Baumannii(50)	46(92)	1(2)	3(6)	7(14)		43(96)	47(94)		3(6)	43(86)	4(8)	3(6)	47(94)		3(6)
Total(50)	46(92)	1(2)	3(6)	7(14)		43(96)	47(94)		3(6)	43(86)	4(8)	3(6)	47(94)		3(6)
A. Baumannii(14)															
A. Baylyi(1)															
A. bereziniae (2)		1(50)						1(50)							
A. Calcoaceticus(1)															
A. dijkshoorniae (2)	1(50)												1(50)		
A. pittii(5)								1(20)							
A. schindleri (1)															
A.tandoi(1)															
Total(27)	1(3.7)	1(3.7)						2(7.4)					1(3.7)		

R: Resistant, I: Intermediate sensitive, S: Sensitive, SXT: Trimethoprim-Sulfamethoxazole, CIP: Ciprofloxacin; TZP: Piperacillin-Tazobactam, TE: Tetracycline, AK: Amikacin, MEM: Meropenem, CL: Colistin, CAZ: Ceftazidime, CN: Gentamycin, IPM, Imipenem

Table 3: The correlation between antibiotic resistance and *adeABC* and *ade*R, *adeS* genes of clinical *A. baumannii* strains (n=50)

Antibiotics		***adeA***		***P***	***adeB***		***P***	***adeC***		***P***	***adeS***		***P***	***adeR***		***P***
		n=7/0	*n=43/1*		*n=2/0*	*n=48/1*		*n=0*	*n=50/1*		*n=16/0*	*n=34/1*		*n=2/0*	*n=48/1*	
		No.(%)			*No.(%)*			*No.(%)*			*No.(%)*			*No.(%)*		
TZP	R	5(71.4)	42(97.7)	0.048	1(50)	46(95.8)	0.118	47(94)	0	N	14(87.5)	33(97.1)	0.237	1(50)	46(95.8)	0.118
	S	2(28.6)	1(2.3)		1(50)	2(4.2)		3(6)	0		2(12.5)	1(2.9)		1(50)	2(4.2)	
CAZ	R	5(71.4)	42(97.7)	0.048	1(50)	46(95.8)	0.118	47(94)	0	N	14(87.5)	33(97.1)	0.237	1(50)	46(95.8)	0.118
	S	2(28.6)	1(2.3)		1(50)	2(4.2)		3(6)	0		2(12.5)	1(2.9)		1(50)	2(4.2)	
CIP	R	5(71.4)	40(93)	0.138	1(50)	44(91.7)	0.192	45(90)	0	N	13(81.3)	32(94.1)	0.311	1(50)	44(91.7)	0.192
	S	2(28.6)	3(7)		1(50)	4(8.3)		5(10)	0		3(18.8)	2(5.9)		1(50)	4(8.3)	
SXT	R	4(57.1)	40(93)	0.029	1(50)	43(89.6)	0.228	44(88)	0	N	12(75)	32(94.1)	0.074	1(50)	43(89.6)	0.228
	S	3(42.9)	3(7)		1(50)	5(10.4)		6(12)	0		4(25)	2(5.9)		1(50)	5(10.4)	
GN	R	4(57.1)	39(90.7)	0.048	1(50)	42(87.5)	0.263	43(86)	0	N	13(81.3)	30(88.2)	0.666	1(50)	42(87.5)	0.263
	S	3(42.9)	4(9.3)		1(50)	6(12.5)		7(14)	0		3(18.8)	4(11.8)		1(50)	6(12.5)	
MEM	R	5(71.4)	41(95.3)	0.089	1(50)	45(93.8)	0.155	46(92)	0	N	13(81.3)	33(97.1)	0.091	1(50)	45(93.8)	0.155
	S	2(28.6)	2(4.7)		1(50)	3(6.2)		4(8)	0		3(18.8)	1(2.9)		1(50)	3(6.3)	
AK	R	4(57.1)	42(97.7)	0.007	1(50)	45(93.8)	0.155	46(92)	0	N	13(81.3)	33(97.1)	0.091	1(50)	45(93.8)	0.155
	S	3(42.9)	1(2.3)		1(50)	3(6.2)		4(8)	0		3(18.8)	1(2.9)		1(50)	3(6.3)	
IMP	R	5(71.4)	42(97.7)	0.048	1(50)	46(95.8)	0.118	47(94)	0	N	14(87.5)	33(97.1)	0.237	1(50)	46(95.8)	0.118
	S	2(28.6)	1(2.3)		1(50)	2(4.2)		3(6)	0		2(12.5)	1(2.9)		1(50)	2(4.2)	
COL	R	0	7(16.3)	0.573	0	7(14.6)	1000	7(14)	0	N	0	7(20.6)	0.081	0	7(14.6)	1000
	S	7(100)	36(83.7)		2(100)	41(85.4)		43(86)	0		16(100)	27(79.4)		2(100)	41(85.4)	

S: Sensitive and intermediate sensitive, R: Resistant, N: Not applicable, *0*: The gene absent, *1*: The gene present, **bold *P*-values** indicate statistically significant correlation. SXT; Trimethoprim-Sulfamethoxazole, CIP; Ciprofloxacin, TZP; Piperacillin-Tazobactam, TE; Tetracycline, AK; Amikacin, MEM; Meropenem, CL; Colistin, CAZ; Ceftazidime, CN; Gentamycin, IPM; Imipenem

Table 4: The correlation between antibiotic resistance and *adeABC,* and *ade*R, *adeS* genes of foodborne *Acinetobacter* spp. (n=27)

Antibiotics		***adeA***		***P***	***adeB***		***P***	***adeC***		***P***	***adeS***		***P***	***adeR***		***P***
		n=4/0	*n=23/1*		*n=18/0*	*n=9/1*		*n=0*	*n=27/1*		*n=17/0*	*n=10/1*		*n=9/0*	*n=18/1*	
		No.(%)			*No.(%)*			*No.(%)*			*No.(%)*			*No.(%)*		
TZP	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	4(100)	23(100)		18(100)	9(100)		0	27(100)		17(100)	10(100)		9(100)	18(100)	
CAZ	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	4(100)	23(100)		18(100)	9(100)		0	27(100)		17(100)	10(100)		9(100)	18(100)	
CIP	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	4(100)	23(100)		18(100)	9(100)		0	27(100)		17(100)	10(100)		9(100)	18(100)	
SXT	R	1(25)	3(13)	0.629	2(11.1)	2(22.2)	0.056	0	4(14.8)	N	1(5.9)	3(30)	0.018	1(11.1)	3(16.7)	0.375
	S	3(75)	20(87)		16(88.9)	7(77.8)		0	22(85.2)		16(94.1)	7(70)		8(88.9)	15(85)	
GN	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	4(100)	23(100)		18(100)	9(100)		0	27(100)		17(100)	10(100)		9(100)	18(100)	
MEM	R	1(25)	0	0.116	1(5.6)	0	1000	0	1(3.7)	N	1(5.9)	0	1000	1(11.1)	0	0.333

	S	3(75)	23(100)		17(94.4)	9(100)		0	26(96.3)		16(94.1)	10(100)		8(88.9)	18(100)	
AK	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	4(100)	23(100)		18(100)	9(100)		0	27(100)		17(100)	10(100)		9(100)	18(100)	
IMP	R	1(25)	0	0.148	1(5.6)	0	1000	0	1(3.7)	N	1(5.9)	0	1000	1(11.1)	0	0.333
	S	3(75)	23(100)		17(94.4)	9(100)		0	26(96.3)		16(94.1)	10(100)		8(88.9)	18(100)	
COL	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	4(100)	23(100)		18(100)	9(100)		0	27(100)		17(100)	10(100)		9(100)	18(100)	

S: Sensitive and intermediate sensitive, R: Resistant, N: Not applicable, *0*: The gene absent, *1*: the gene present, **bold *P*-values** indicate statistically significant correlation. SXT; Trimethoprim-Sulfamethoxazole, CIP; Ciprofloxacin, TZP; Piperacillin-Tazobactam, TE; Tetracycline, AK; Amikacin, MEM; Meropenem, CL; Colistin, CAZ; Ceftazidime, CN; Gentamycin, IPM; Imipenem

Discussion

Acinetobacter spp. can be found in various foods but, the multidrug-resistant *A. baumannii* (MDR-AB) is a crucial problem in the medical field because it is difficult to treat and causes fatal results (16,17). Most of our foodborne and clinical strains was *A. baumanni* (14, 51.9%; 50, 100%); *A. pitti* (5) and *A. calcoaceticus* (1) were also considered *A. baumannii* group that is most frequently associated with nosocomial infections worldwide (6,18). In our foodborne isolates, this group's rate was at a frequency of 74.07% (20/27). Furthermore, 7 (25.9%) of foodborne strains were found to be resistant to trimethoprim-sulfamethoxazole, tetracycline, meropenem and imipenem, and *A. dijkshoorniae* strain was found MDR (Table 2). Our results (3.7%; 1/27, MDR) were lower than previous studies (6,19). They found about 29.8% of the strains were (MDR) and 4.4% as (XDR) of *Acinetobacter* spp. in fruit and vegetable samples in Portugal, and 50% (MDR) in chicken meat samples in Iran. Our clinical *A. baumanni* strains were nearly fully resistant (95%) to the antibiotics tested and (92%; 8%) of them XDR and MDR respectively. These results were lower than previous results for countries; Iran (100%), China (100%), and Pakistan (16,20-22). Additionally, seven (14%) clinical strains were found resistant to colistin, and their MIC value was ≥4 μg/mL, and these strains were resistant to all tested antibiotics. Our result is higher than the resistance rate (2.9%) reported from Iran (22), and unlike previous studies in Pakistan and Saudi Arabia, no colistin-resistant *A. baumannii* isolates were found in their studies (16,21).

The multidrug efflux pump for the trans membrane protein of adeABC is encoded by the *adeB* gene. Nearly all clinical *A. baumannii* isolates (96%), and 33% of foodborne *Acinetobacter* spp. in the present study were found to carry the *adeB* gene. This rate was 100% in Iran (23). The disruption of this gene leads to the loss of multidrug resistance previously reported (24). Our study showed a high incidence of *adeA, adeB, and adeC,* genes (86%, 96%, and 100%) respectively among clinical isolates (Fig. 1). Similar results were reported (88.5%, 100%, and 93.9%, respectively) in Iran (23). There was a significant difference in the occurrence of multi-efflux pump genes between human clinical and foodborne isolates ($P<0.001$). Multidrug efflux pumps can play a crucial role in the mechanism of resistance in our clinical strains in agreement with a recent report conducted in Iran (23).

Antibiotic resistance and *adeR* and *adeS* genes incidence of clinical *A. baumannii* strains have shown some differences from country to country. The distribution of *adeS*, and *adeR* genes among our *A. baumannii* strains was 68%, and 96%, respectively. Our *adeS* gene rate was lower but *adeR* gene prevalence was higher than the previous studies in Iran and China (25,26).

Antibiotic-sensitive *Acinetobacter* spp. could be carry *adeA, adeB, adeC* and *adeR, adeS* genes, but some of them implied that only resistant strains carried those genes (11). However, we detected a higher incidence of these genes, (%85.2; 33.3%; 100 and 37.3%; 66.7% respectively) in our food-

borne strains, while being sensitive to most of the tested antibiotics.
In foodborne *Acinetobacter* spp., a positive correlation was found between *adeS* gene positivity and trimethoprim-sulfamethoxazole resistance (*P*=0.018). *adeS* gene may have an effective role in acquiring trimethoprim-sulfamethoxazole (folate pathway inhibitors) resistance in foodborne *Acinetobacter* spp. (Table 4). There was a significant interdependence between harboring of *adeA* gene positivity and amikacin, piperacillin-tazobactam, ceftazidime, gentamycin, imipenem, and trimethoprim-sulfamethoxazole resistance that were found respectively in clinical *Acinetobacter* spp. (Table 3). The presence of *adeABC* genes can stimulate the resistance to piperacillin-tazobactam, ceftazidime, gentamycin, imipenem, and trimethoprim-sulfamethoxazole resistance, and drug efflux pumps contribute to the resistance to β-lactam/β-lactamase, cephems, aminoglycosides, carbapenems, and folate pathway inhibitors, respectively in clinical strains. Our results show some similarities to previous reports (27) about *adeA* and *adeS* genes are related to the tetracycline, ciprofloxacin, gentamicin, and amikacin resistance and another findings of stimulation of carbapenem resistance (11). Our findings digress from previous reports claiming that the *adeC* gene is not essential for (AB-MDR) phenotypes. We have detected *adeC* (100%) gene in all strains (sensitive and resistant) (Fig. 1). Besides, our results are in line with some previous studies which revealed the *adeABC* efflux pump was present in both carbapenem-resistant and sensitive strains (28,29).

Conclusion

High rates of efflux pump genes in many antibiotic-susceptible food isolates suggests that these genes may have been transferred by other pathogens in the food processing line. The positive correlation between the *adeS* gene positivity and trimethoprim-sulfamethoxazole resistance in them is a crucial finding. Moreover, the positive correlation between the phenotypic resistance to various antibiotics of clinical *A. baumannii* strains carrying the *adeA* gene be taken into consideration in terms of the effect of the *adeA* gene on the development of carbapenems and other antibiotics' phenotypic resistance. No genetic relations were found between the two groups, which meant that foodborne *Acinetobacter* spp. was not a reason for *A. baumanni* infections. However, more foodborne and clinical strains should be studied in this regard in a wider area for more information.

Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This study was supported by the Osmaniye Korkut Ata University Scientific Research Project Unit (project number: OKUBAP-2020-PT 2-001).

Conflict of interest

The authors declare that there is no conflict of interests.

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