The presence of antibiotic resistance and molecular characterization of aminoglycoside and *PmrA* genes among food- and clinical-acquired *Acinetobacter* isolates

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ABSTRACT

The assessment of antibiotic resistance and related genes of foodborne *Acinetobacter* spp. and the analysis of whether they are genetically related to clinical infection-agent strains are crucial in terms of sustainability of food safety. The study at hand investigated antibiotic resistance, aminoglycoside-modifying enzyme (AME), and colistin resistance (*PmrA*) genes, clonal relationships while evaluating a possible correlation between antibiotic resistance and related genes between 27 foodborne and 50 clinical *Acinetobacter* spp. in Turkey. Antimicrobial susceptibilities, AME, *PmrA* genes, and clonal relatedness of the strains were performed by disc diffusion, PCR, and Pulsed Field gel Electrophoresis (PFGE) methods, respectively. The *aph-AI*, *aph-6*, *anth(3'')-I*, *aadA1*, *aadB,* and *PmrA* genes were found as 48%(n=24), 22%(n=11), 14%(n=7), 2%(n=1), 4%(n=2), and 92%(n=46) respectively, in clinical strains. This rate was found as 51.9%(n=14),59.3%(n=16), 70.4%(n=19), 7.4%(n=2), 0%(n=0), and 100%(n=27), respectively in foodborne isolates. A positive correlation existed between the number of *aph-AI* gene positivity and trimethoprim-sulfamethoxazole and gentamycin resistance; *anth (3'')-I* gene positivity, and colistin resistance; *PmrA* gene positivity and piperacillin-tazobactam, ceftazidime, meropenem, amikacin, and imipenem resistance in clinical strains ($P<0.05$). A positive correlation between trimethoprim-sulfamethoxazole resistance and *aadAI* gene positivity was found in foodborne strains ($P<0.05$). Clonal relations were absent between foodborne and clinical *A. baumanni* species. Finally, AME genes rise parallel to multidrug-resistance in the clinical isolates, and foods may be potential reservoirs for disseminating multi-AME and *PmrA* genes while being susceptible to several antibiotics.

Introduction

Acinetobacter baumannii is a Gram-negative bacterium that can survive in harsh conditions both in nature, various food animals/poultry meat, dairy products, fruit/vegetables, and in the human body. A widespread/inappropriate application of broad-spectrum antibiotics in the medical field, agriculture, and veterinary area was conducted. As a result, multidrug-resistant *Acinetobacter* species that cause clinical nosocomial infections (bacteremia, pneumonia, meningitis, and urinary tract infections) have emerged because of fatal effects and economical losses around the world. Therefore, this bacterium is considered one of the six dangerous microorganisms by the Diseases Society of America. In the last decades, antibiotic resistance is an increasing problem worldwide, causing failures in the treatment of infections that affect not only hospital infections but also public health (19, 23, 30, 34). In *Acinetobacter* infections (humans and animals), beta-lactam group antibiotics are used for bactericidal effects during the lag period of the bacteria. In the resting period of the bacteria, aminoglycosides (gentamycin, tobramycin, and cephemycin) are used in combination with beta-lactam group drugs (imipenem and meropenem) due to their synergistic

effects. However, imipenem-resistant *A. baumannii* has become a worldwide problem due to the bacterial production of β-lactamase encoded by the bla_{OXA-23} gene, which is also carried by foodborne *Acinetobacter* spp. as previously reported. However, there is less information on AME and colistin resistance genes carried by foodborne *Acinetobacter* spp. (17, 20). There are two main mechanisms of Gram-negative bacteria resistance to aminoglycosides. Firstly, aminoglycosides entering the bacteria are modified by aminoglycoside-modifying enzymes and become dysfunctional. Secondly, the methylation of target sites is induced by 16S rRNA methylases, resulting in decreased affinity of 16S rRNA for the antibiotic (7). With the inclusion of variant acetyltransferases [aac(3)-I, aac(3)-II, aac(3)-III, aac(6')-I, aac(6')-II, aac(6')-III], phosphotransferases [aph(3')-I, aph(3')-II, aph(3')-VI], and the nucleotidyltransferases [ant(3-)-I, ant(4')-I, and ant(2'')-I], AMEs have been identified in *Acinetobacter* spp. Aminoglycoside resistance in *Acinetobacter* species is mostly due to the production of *aph-AI, aph-6, anth(3'')-I, aacC1, aadA1, aadB* genes responsible for AME enzymes by *Acinetobacter* spp. (4, 15, 19, 33).

Colistin is an antibiotic of last-line drug used in the treatment of *A. baumannii* infections. Colistin resistance is considered a serious problem, due to a lack of alternative antibiotics. The main mechanism of colistin resistance in *Acinetobacter baumannii* is the addition of a cationic group to the lipopolysaccharide layer of the bacteria; the complete loss of lipopolysaccharide production leads to resistance. The addition of a cationic group in *A. baumannii* in the *PmrAB*gene region depends on mutation (6, 27). The development of acquired antibiotic resistance in food-borne and clinical-*Acinetobacter* strains and the genes encoding AME enzymes and 16S rRNA methylase should be investigated, whether food-borne strains play a role in the spread of these genes and whether they are genetically related to clinical infection agent strains (5, 19, 21, 29). Several types of research have gone into the resistance mechanisms and genes in *Acinetobacter* spp. However, in comparison, there are so few studies on the spread of those genes due to their presence in the food chain (5, 17, 23).Investigation of phenotypic and genotypic resistance to antibiotics in *A. baumannii* strains may have global implications for the maintenance of antimicrobial chemotherapy (15). The high prevalence of AME and *PmrA* genes associated with phenotypic resistance makes it possible to choose the most accurate antibiotics in agriculture, veterinary and medical fields; it is so crucial in establishing the best policies to prevent the spread of genes encoding resistance (5, 15, 17, 23). However, there is a lack of studies about this issue in Middle East Countries (15) including Turkey. The relationship between mentioned encoding genes and phenotypic resistance analyses in our country/Middle East is a gap that this study aims to fill.

For this purpose; [1] five aminoglycoside-modifying enzymes (*aph-AI, aph-6, anth(3'')-I, aadA1, aadB*) and *PmrA* genes were evaluated in 27 foodborne *Acinetobacter* spp. and 50 *A. baumannii* strains of clinical originated. [2] A possible correlation between the aminoglycoside genes range and antibiotic resistance was statistically examined in all strains. [3] The analysis also answered the question as to whether genetically related *A. baumanni* species of food and clinical origin were investigated with the PFGE genetic comparison method, which is considered ''gold standard'' to discrimination of endemic strains. This method is accepted as a solid standard with high discrimination power in comparison of endemic species and other species. To the best of our knowledge, this is the first report of molecular characterization of antimicrobial-resistant *Acinetobacter* spp. from various foods and clinical samples in our country.

Materials and Methods

Place and sampling: In this study, a total of 250 samples were analyzed for the presence of *Acinetobacter* spp. A total of 102 food samples [n=25 cheese, n=39 fruit (banana, strawberries, tomatoes) and vegetables (lettuces, packed salads, cabbages), n=17 sucuk, n=8 veal, and n=13 chicken meat] were purchased from 12 markets in two provinces of southern Turkey. Clinical strains isolated from 148 clinical samples obtained from two hospitals in Adana and Mersin provinces were sent to Çukurova University, Clinical Microbiology Laboratory for a 4-year period (2018-2021). A total of 50 clinical strains (non-repetitive) were collected. Half of the strains were collected from female patients (25, 50%). The age ± standard deviation of the patients was 65.9± 15.1. Since the PFGE method can provide meaningful data for the analysis of short-term outbreaks, food isolates were collected in a similar period from the markets in the close location (campus) of the hospitals where the clinical isolates were collected.

***Isolation and identification of Acinetobacter spp.*:** The isolation of foodborne isolates was performed, as described previously (5, 23). The samples were cultured on Tryptone Soy Agar (TSA) plates containing 5% sheep blood (Oxoid) media at 37°C for 24 hours. The isolates were first evaluated by Gram staining, motility, catalase production, and the oxidase test to examine the morphology of colonies and biochemical test characteristics (7). To identify the presumptive colonies, a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF) (Bruker, Germany)

was used (10). Columbia and Macconkey agars (Becton-Dickinson, Sparks, MD), were used to grow the clinical isolates. To obtain pure cultures, blood agar [containing 5% sheep blood (Oxoid)] was used and identified by (MALDI-TOF) system. All isolates were also confirmed by BBL Crystal E/NF test kit (Becton Dickinson, Australia) in the Microbiology Laboratory of Medical Faculty. The isolates were confirmed by the PCR method in terms of *bla*$_{OXA-51}$ gene carriage (35). Brain Hearth Broth medium (BHI) (including 10% of glycerol and10% of human blood) was used as a storage medium at -20ºC covering a genetic analysis of all identified species.

Detection of the* AME *and PmrA genes: The genomic DNA was extracted with the boiling method as previously described (13). A spectrophotometer was used to measure the extracted DNA (100 ng/µL DNA for each sample) (UV-VIS Spectrophotometer CHIBIOS). The DNAs were stored at -20ºC before genotypic tests were performed. A multiplex PCR protocol was performed to screen specific *aph-AI*, *aph-A6, ant(''3)-I*, *aadB, aadAI,* and colistin resistance genes (*PmrA*) as described before (2, 16, 35, 37). The PCR conditions and the list of specific primers were presented in Table 1.The amplicons were run on 1.5% agarose gel [PegGOLD Universal Agarose, 91052 Erlangen Deutschland, 2%(w/v)], which was visualized on a UV transilluminator (Kodak, New York, USA).

Antibiotic susceptibility test: The antimicrobial susceptibility test was performed through a disk diffusion method on Mueller-Hinton Agar (Merck KGaA, Germany)(7). The test was performed with nine antibiotic discs; ciprofloxacin (CIP, 5µg), piperacillin-tazobactam (TZP, 100/10µg), ceftazidime (CAZ, 30µg), imipenem (IPM, 10µg), meropenem (MEM, 10µg), amikacin (AK, 30 µg), trimethoprim/sulfamethoxazole (SXT,10 µg), gentamicin (CN,10 µg), and tetracycline (TE,30 µg), (all discs from Oxoid). The susceptibility was interpreted by Clinical and Laboratory Standards Institute (CLSI)) guidelines (7). A reference strain was obtained by using an index strain of *A. baumannii* (CI-74) as an infection agent. It was collected from the Medical Microbiology Laboratory. Colistin (CL) susceptibility and MIC value of the strains were determined by the agar dilution method according to EUCAST guidelines (7, 12).*Escherichia coli* ATCC 25922 was usedas a quality control (QC) strain for susceptibility tests. Isolates with a MIC ≤2 mg/L for colistin were accepted as susceptible (7, 12).

Table 1. The list of primers and amplification conditions used in this study.

Genes	Primer sequences (5'-3')	Product size (bp)	Annealing (˚C)	Amplification conditions	Reference
bla$_{OXA-51}$	GACCGAGTATGTACCTGCTTCGACC	497	55	The initial cycle of 94˚C for 4 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, 72˚C for 1 min; 1 cycle of 72˚C for 7 min.	(35)
	GAGGCTGAACAACCCATCCAGTTAACC				
ant(3'')-I	TGATTTGCTGGTTACGGTGAC	284	55	The initial cycle of 94ºC for 3 min.; 40 cycles of: 94ºC for 30 s, 55ºC for 40s,72ºC for 40 s;1 cycle: of 72ºC for 10 min.	(37)
	CGCTATGTTCTCTTGCTTTTG				
aph-AI	ACAGAAGAGCTGCAGGAAATG	623	55	The initial cycle of 94ºC for 3 min.; 40 cycles of: 94ºC for 30 s, 55ºC for 40s,72ºC for 40 s;1 cycle: of 72ºC for 10 min.	(16)
	GACTGACGTCCAAGTTCCCAA				
aph-A6	GCACGCTATTACCAACTATGA	736	55		
	TAAGAAAGAACATCACCACGA				
aadAI	AGATTTCATCTTTGATTCTTGG	624	62	The initial cycle of 94ºC for 3 min.; 40 cycles of: 94ºC for 30 s, 62ºC for 40s,72ºC for 40 s;1 cycle: of 72ºC for 10 min.	
	AATTGATTCTTAGCATCTGG				
aadB	ACTCGGGGATTGATAGGC	495	68	The initial cycle of 94ºC for 3 min.; 40 cycles of: 94ºC for 30 s, 68ºC for 40s,72ºC for 40 s;1 cycle: of 72ºC for 10 min.	
	GCTGCTAAAGCTGCGCTT				
PmrA	TCTGCAATAGAGATAGCCGC3	175	57	The initial cycle of 94ºC for 3 min.; 40 cycles of: 94ºC for 30 s, 57ºC for 40s,72ºC for 40 s;1 cycle: of 72ºC for 10 min.	(2)
	GGAGTAGCTATCCCAGCATT				

PFGE analysis: Plug preparation, lysis, cell washing, restriction digestion, and electrophoresis were performed as previously described (8). PFGE was run in a CHEF-DR II apparatus (Bio-Rad, The USA), with pulses ranging from 5 to 30s at a voltage of 6 V/cm at 12°C for 20 h. Products were visualized after staining with ethidium bromide (50 µg/mL) and photographed. Gel images were exported to Gelcompar II software (version 3.0; Applied Maths, Sint Martens Latem, Belgium) for analysis. Comparisons were made by way of the band-based Dice coefficient. Dendrograms were generated by using the unweighted pair group method based on the arithmetic averaging method with a 1.5% position tolerance. Isolates were considered to be closely related if the Dice coefficient correlation was ≥80%.

Statistical Analysis: The statistical program S-PLUS 20 (S-PLUS 20.00 for Windows, Professional Edition) was used for data analysis. The number and percentage of categorical measurements were summarized. The Chi-square test was used to compare AME/*PmrA* genes and antibiotic susceptibility. Fisher's exact test and Mann-Whitney U tests were used where appropriate. A P value of <0.05 was considered statistically significant.

Results

A total of 27 foodborne isolates were identified as the following species; *A. baumannii* (n=14), *A. pittii* (n=5), *A. bereziniae* (n=2), *A. dijkshoorniae* (n=2), *A. calcoaceticus* (n=1), *A. baylyi* (n=1), *A. schindleri* (n=1), and *A. tandoii* (n=1). A total of 50 clinical isolates were identified as *A. baumannii*. The majority of the clinical isolates in a nosocomial environment were from intensive care internal medicine (*n*=17, 34%), nephrology/urology, (*n*=9, 18%), burn unit (*n*=6, 12%), and other clinics (general surgery anesthesia and brain surgery intensive care) (*n*=14, 28%), respectively. Clinical strains were isolated from aspiration fluid (*n*=14, 28%), wound (*n*=11, 22%), sputum (*n*=10, 20%), blood (*n*=8, 16%), and urine (*n*=7, 14%) samples, respectively. Distribution and antibiotic susceptibilities of the foodborne and clinical strains were presented in Table 2. The MIC value of clinical XDR (*n*=46, 92%), and MDR (*n*=4, 8%) strains were found between 64-128 µg/mL. All colistin-resistant strains' MIC value was ≥4 µg/mL. A summary of the incidence of the$bla_{\text{OXA-51}}$, AME, and *PmrA* genes among the *Acinetobacter* spp. and their origin was presented in Table 3. The gel image of $bla_{\text{OXA-51}}$ and AME genes of different sources was presented in Figure1. A summary of correlations between the AME, *PmrA* genes, and antibiotic resistance patterns of clinical and foodborne *Acinetobacter* spp. was presented in Tables 4 and 5.

PFGE results indicated a total of 34 *A. baumannii* isolates formed 24 different pulsotypes. The width of the clusters varied between 2–3 strains; a total of 6 groups were included 2 members such as“d, l, m, n, p, t”. Two groups were included 3 members as “j, s”. The “s” group has 100% similar clusters (s1). It was noted that there were 8 different PFGE groups. The clustering rate was calculated as 52.9. A total of 16 strains were unique, and 18 (52.9%) strains were collected in 8 closely related groups. There was no clonal association between foodborne and clinical strains. Foodborne and clinical isolates from different genetically unique/related groups among themselves (Figure 2**).**

Table 2. Antimicrobial susceptibility profile of all *Acinetobacter* spp. (*n*=77).

	Resistance Patterns			No (%)						
Isolates	**A**/SXT	**B**/CIP/	**C**/TZP	**D**/TE	**E**/AK	**F**/MEM	CL	**G**/CAZ	**E**/CN	**F**/IMP
Foodborne strains (27)										
A. baumannii(14)	2(14.3)*			2(14.3)						
A. calcoaceticus(1)										
A.bereziniae(2)						1(50)*		1(50)*		
A. baylyi(1)										
A.dijkshoorniae(2)	1(50)			1(50)		1(50)				1(50)
A.pittii(5)	1(20)							1(20)*		
A.tandoi(1)										
A. schindleri(1)										
Total	4(14.8)			3(11.1)		2(7.4)		2(7.4)*		1(3.7)
Clinical Strains(50)										
A. baumannii (50)	44(88)	45(90)	47(94)		**46(92)**	46(92)	**7(14)**	47(94)	**43(86)**	47(94)
Total	44(88)	45(90)	47(94)		**46(92)**	46(92)	**7(14)**	47(94	**43(86)**	47(94)

*: Intermediately sensitive; CIP, Ciprofloxacin; SXT, Trimethoprim-Sulfamethoxazole; TE, Tetracycline; CL, Colistin; TZP, Piperacillin-Tazobactam; CAZ, Ceftazidime; IPM, Imipenem; CN. Gentamycin; AK, Amikacin; MEM, Meropenem. **A:** Folate pathway inhibitors, **B:** Fluoroquinolones, **C:** β-lactam/β-lactamase inhibitor combinations, **D:** Tetracyclines, **E: Aminoglycosides**, **F:** Carbapenems, **G:** Cephems.

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Table 3. Distribution of *bla*$_{OXA-51}$, aminoglycoside, and *PmrA* resistances genes of *Acinetobacter* spp. in the present work.

	Resistance genes						No (%)
Isolates	***bla*$_{OXA-51}$**	***aph-AI***	***aph-6***	***anth(3'')-I***	***aadA1***	***aadB***	***PmrA***
Foodborne strains (**27**)							
A. baumannii(14)	14(100)	9(64.3)	9(64.3)	9(64.3)	2(14.3)	0	14(100)
A. baylyi(1)	0	0	0	0	0	0	1(100)
A. bereziniae (2)	0	1(50)	1(50)	2(100)	0	0	2(100)
A. calcoaceticus(1)	0	1(100)	0	1(100)	0	0	1(100)
A. dijkshoorniae(2)	0	1(50)	1(50)	1(50)	0	0	2(100)
A. pittii(5)	0	2(40)	5(100)	4(80)	0	0	5(100)
A. schindleri(1)	0	0	0	1(100)	0	0	1(100)
A. tandoi(1)	0	0	0	1(100	0	0	1(100)
Total	14(51.9)	14(51.9)	16(59.3)	19(70.4))	2(7.4)	0	27(100)
Clinical Strains (**50**)							
A. baumannii (**50**)	50(100)	24(48)	11(22)	7(14)	1(2)	2(4)	46(92)
Total	50(100)	24(48)	11(22)	7(14)	1(2)	2(4)	46(92)

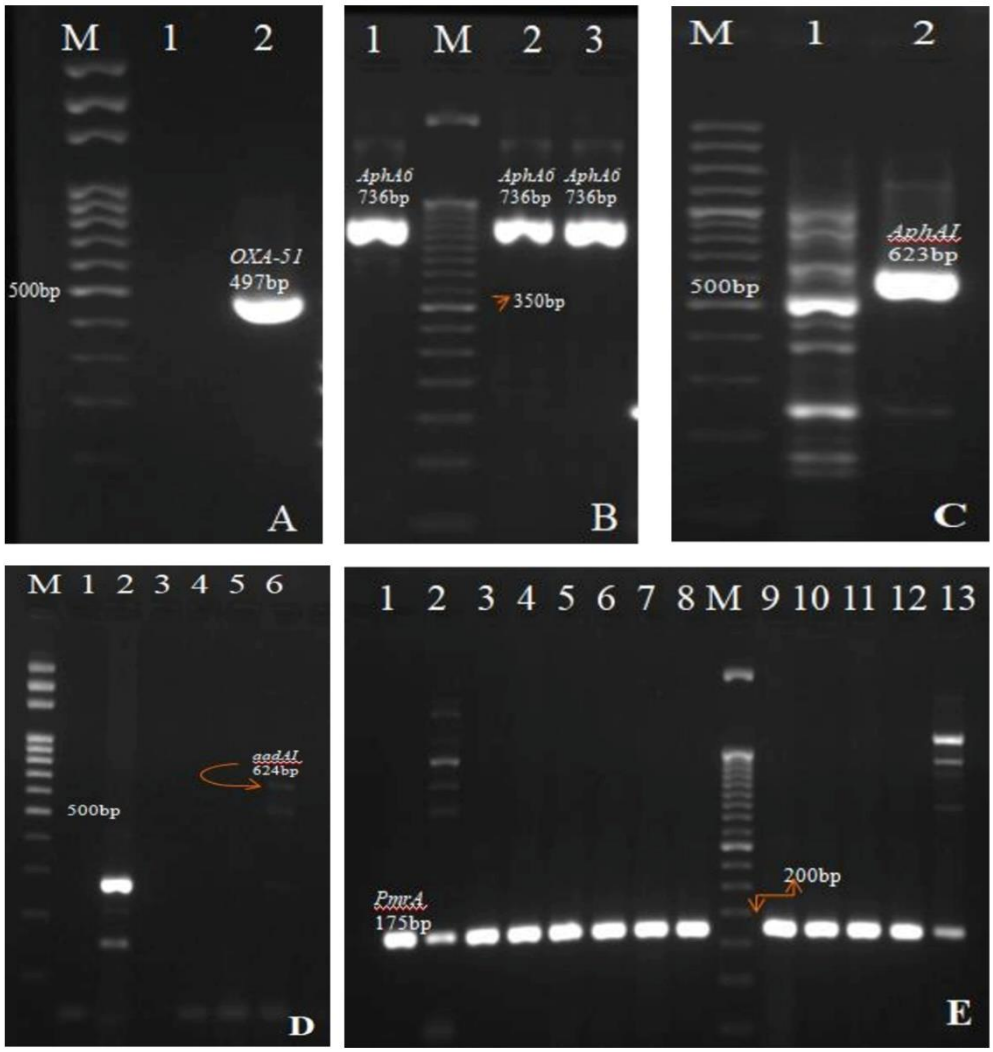

Figure 1. Agarose gel electrophoresis of PCR-amplified products of *Acinetobacter* spp.
(A)-(*bla*$_{OXA-51}$); M: Marker (100bp), 1: Negative control, 2: *A. baumannii*(lettuce). **(B)**-(*aph-A6)*;1: *A. baumannii*(veal), M: Marker(50bp), 2: *A. baumannii*(sucuk), 3: *A. schindleri*(cheese). **(C)**-(*aph-AI)*;M: Marker (100bp), 1: *A. baumannii*(purple cabbage), 2: *A. baumannii*(cheese). **(D)**-(*aadAI*); M: Marker (100bp), 1: *A. pitti* (cheese), 2: *A. pittii* (packet salad), 3: *A. bereziniae*(veal), 4: *A. dijkshoorniae*(packet salad), 5: *A. baumannii*(packet salad), 6: *A. baumannii*(lettuce). **(E)**-(*PmrA*); 1: *A. pittii*(cheese), 2: *A. bereziniae*(veal), 3: *A. dijkshoorniae*(packet salad), 4: *A.pittii* (packet salad), 5: *A. baumannii*(packet salad), 6: *A. baumannii*(lettuce), 7: *A. baumannii*(lettuce) 8: *A. baumannii*(spinach), M: Marker (50bp), 9: *A. baumannii*(purple cabbage), 10: Control strain (*A. baumannii*), 11: *A. pittii* (packet salad), 12: *A. baumannii*(cheese), 13: *A. baumannii*(traditional cheese).

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Ankara Univ Vet Fak Derg, 71 • 2, 2024

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Table 4. Antibiotic susceptibility and resistant genes relations in clinical *Acinetobacter* spp. strains (*n*=50) in the present study.

		Resistance genes No (%)																			
Tested Antibiotics		***aph-AI***		***P***	***aph-6***		***P***	***anth(3'')-I***		***P***	***aadA1***		***P***	***aadB***		***P***	***PmrA***		***P***
		n=26/0[a]	***n=24/1[b]***		***n=39/0[a]***	***n=11/1[b]***		***n=43/0[a]***	***n=7/1[b]***		***n=49/0[a]***	***n=1/1[b]***		***n=48/0[a]***	***n=2/1[b]***		***n=4/0[a]***	***n=46/1[b]***	
		No (%)			**No (%)**			**No (%)**			**No (%)**			**No (%)**			**No (%)**		
TZP	**R**	23(88.5)	24(100)		36(92.3)	11(100)		40(93)	7(100)		46(93.9)	1(100)		45(93.8)	2(100)		2(50)	45(97.8)	
	S	3(11.5)	0	0.236[c]	3(7.7)	0	1000[c]	3(7)	0	1000[c]	3(6.1)	0	1000[c]	3(6.3)	0	1000[c]	2(50)	1(2.2)	0.014[c]
CAZ	**R**	23(88.5)	24(100)		36(92.3)	11(100)		40(93)	7(100)		46(93.9)	1(100)		45(93.8)	2(100)		2(50)	45(97.8)	
	S	3(11.5)	0	0.236[c]	3(7.7)	0	1000[c]	3(7)	0	1000[c]	3(6.1)	0	1000[c]	3(6.3)	0	1000[c]	2(50)	1(2.2)	0.014[c]
CIP	**R**	21(80.8)	24(100)		36(92.3)	9(81.8)		38(88.4)	7(100)		44(89.8)	1(100)		43(89.6)	2(100)		2(50)	43(93.5)	
	S	5(19.2)	0	0.051[c]	3(7.7)	2(18.2)	0.301[c]	5(11.6)	0	1000[c]	5(10.2)	0	1000[c]	5(10.4)	0	1000[c]	2(50)	3(6.5)	0.045[c]
SXT	**R**	20(76.9)	24(100)		33(84.6)	11(100)		38(88.4)	6(85.7)		43(87.8)	1(100)		42(87.5)	2(100)		2(50)	42(91.3)	
	S	6(23.1)	0	0.023[c]	6(15.4)	0	0.317[c]	5(11.6)	1(14.3)	1000[c]	6(12.2)	0	1000[c]	6(12.5)	0	1000[c]	2(50)	4(8.7)	0.066[c]
CN	**R**	19(73.1)	24(100)		33(84.6)	10(90.9)		37(86)	6(85.7)		42(85.7)	1(100)		41(85.4)	2(100)		2(50)	41(89.1)	
	S	7(26.9)	0	0.010[c]	6(15.4)	1(9.1)	1000[c]	6(14)	1(14.3)	1000[c]	7(14.3)	0	1000[c]	7(14.6)	0	1000[c]	2(50)	5(10.9)	0.089[c]
MEM	**R**	22(84.6)	24(100)		35(89.7)	11(100)		39(90.7)	7(100)		45(91.8)	1(100)		44(91.7)	2(100)		2(50)	44(95.7)	
	S	4(15.4)	0	0.111[c]	4(10.3)	0	0.563[c]	4(9.3)	0	1000[c]	4(8.2)	0	1000[c]	4(8.3)	0	1000[c]	2(50)	2(4.3)	0.028[c]
AK	**R**	22(84.6)	24(100)		35(89.7)	11(100)		39(90.7)	7(100)		45(91.8)	1(100)		44(91.7)	2(100)		2(50)	44(95.7)	
	S	4(15.4)	0	0.111[c]	4(10.3)	0	0.563[c]	4(9.3)	0	1000[c]	4(8.2)	0	1000[c]	4(8.3)	0	1000[c]	2(50)	2(4.3)	0.028[c]
IMP	**R**	23(88.5)	24(100)		36(92.3)	11(100)		40(93)	7(100)		46(93.9)	1(100)		45(93.8)	2(100)		2(50)	45(97.8)	
	S	3(11.5)	0	0.236[c]	3(7.7)	0	1000[c]	3(7)	0	1000[c]	3(6.1)	0	1000[c]	3(6.3)	0	1000[c]	2(50)	1(2.2)	0.014[c]
CL	**R**	2(7.7)	5(20.4)		5(12.8)	2(18.2)		4(9.3)	3(42.9)		7(14.3)	0		6(12.5)	1(50)		0	7(15.2)	
	S	24(92.3)	19(79.2)	0.239[c]	34(87.2)	9(81.8)	0.641[c]	39(90.7)	4(57.1)	0.048[c]	42(85.7)	1(100)	1000[c]	42(87.5)	1(50)	0.263[c]	4(100)	39(84.8)	0.536[c]

0[a]: No gene, *1[b]*: Gene present, ***c*:** fisher`s exact test, R: Resistant, S: Sensitive and intermediate sensitive; TZP, Piperacillin-Tazobactam; CAZ, Ceftazidime; CIP, Ciprofloxacin; SXT, Trimethoprim-Sulfamethoxazole; CN, Gentamycin; MEM, Meropenem; AK, Amikacin; IPM, Imipenem; CL, Colistin. The significant correlations were indicated in ***bold P*** value.

Table 5. Antibiotic susceptibility and resistant genes relations in foodborne *Acinetobacter* spp. (*n*=27) in this study.

Tested Antibiotics		Resistance genes No (%)																
		aph-AI		*P*	*aph-6*		*P*	*anth(3'')-I*		*P*	*aadA1*		*P*	*aadB*		*P*	*PmrA*		*P*
		n=13/0[a]	*n=14/1*[b]		*n=11/0*[a]	*n=16/1*[b]		*n=8/0*[a]	*n=19/1*[b]		*n=25/0*[a]	*n=2/1*[b]		*n=27/0*[a]	*n=0/1*[b]		*n=0/0*[a]	*n=27/1*[b]	
		No (%)			No (%)			No (%)			No (%)			No (%)			No (%)		
TZP	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	13(48.1)	14(51.9)		11(100)	16(100)		8(100)	19(100)		25(100)	2(100)		27(100)	0		0	27(100)	
CAZ	R	1(7.7)	1(7.1)	1000[c]	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	12(92.3)	13(92.9)		11(100)	16(100)		8(100)	19(100)		25(100)	2(100)		27(100)	0		0	27(100)	
CIP	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	13(48.1)	14(51.9)		11(100)	16(100)		8(100)	19(100)		25(100)	2(100)		27(100)	0		0	27(100)	
SXT	R	2(15.4)	2(14.3)	0.249[c]	1(9.1)	3(18.8)	0.761[c]	0	4(21.1)	0.191[c]	3(12)	1(50)	**0.030**[c]	4(14.8)	0	N	0	4(14.8)	N
	S	11(84.6)	12(85.7)		10(90.9)	13(81.2)		8(100)	15(78.9)		22(88)	1(50)		23(85.2)	0		0	23(85.2)	
CN	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	13(48.1)	14(51.9)		11(100)	16(100)		8(100)	19(100)		25(100)	2(100)		27(100)	0		0	27(100)	
MEM	R	1(7.7)	0	0.481[c]	1(9.1)	0	0.407[c]	0	1(5.3)	1000[c]	1(4)	0	1000[c]	1(3.7)	0	N	0	1(3.7)	N
	S	12(92.3)	14(100)		10(90.9)	16(100)		8(100)	18(94.7)		24(96)	2(100)		26(96.3)	0		0	26(96.3)	
AK	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	13(48.1)	14(51.9)		11(100)	16(100)		8(100)	19(100)		25(100)	2(100)		27(100)	0		0	27(100)	
IMP	R	1(7.7)	0	0.481[c]	1(9.1)	0	0.407[c]	0	1(5.3)	1000[c]	1(4)	0	1000[c]	1(3.7)	0	N	0	1(3.7)	N
	S	12(92.3)	14(100)		10(90.9)	16(100)		8(100)	18(94.7)		24(96)	2(100)		26(96.3)	0		0	26(96.3)	
CL	R	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N	0	0	N
	S	13(48.1)	14(51.9)		11(100)	16(100)		8(100)	19(100)		25(100)	2(100)		27(100)	0		0	27(100)	

0[a]: No gene, *1*[b]: Gene present, ***c*:** fisher`s exact test, R: Resistant, S: Sensitive and intermediate sensitive, N: No applicable; TZP, Piperacillin-Tazobactam; CAZ, Ceftazidime; CIP, Ciprofloxacin; SXT, Trimethoprim-Sulfamethoxazole; CN, Gentamycin; MEM, Meropenem; AK, Amikacin; IPM, Imipenem; CL, Colistin**.** The significant correlations were indicated in ***bold P*** value.

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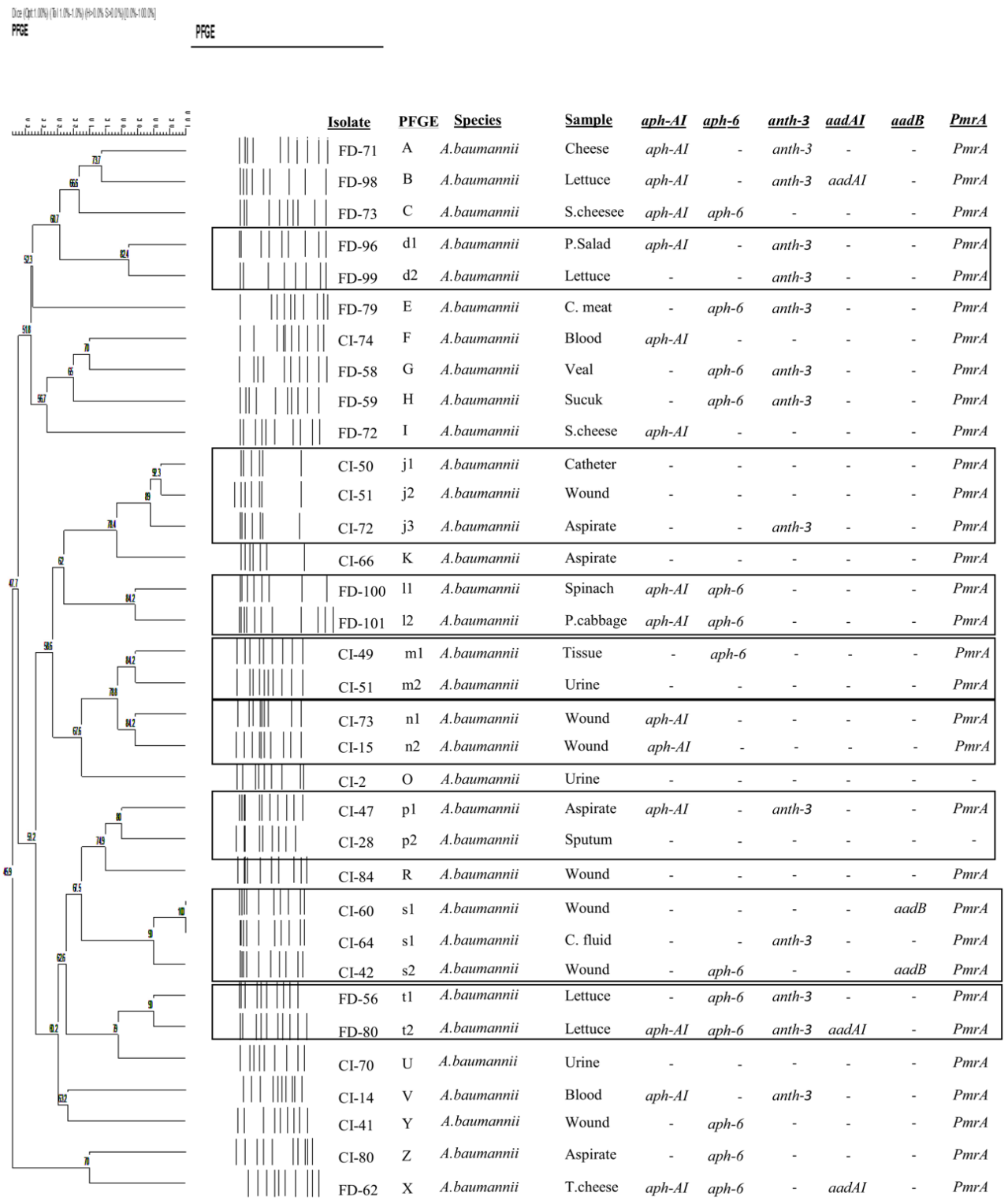

Isolate	PFGE	Species	Sample	*aph-AI*	*aph-6*	*anth-3*	*aadAI*	*aadB*	*PmrA*
FD-71	A	*A.baumannii*	Cheese	*aph-AI*	-	*anth-3*	-	-	*PmrA*
FD-98	B	*A.baumannii*	Lettuce	*aph-AI*	-	*anth-3*	*aadAI*	-	*PmrA*
FD-73	C	*A.baumannii*	S.cheesee	*aph-AI*	*aph-6*	-	-	-	*PmrA*
FD-96	d1	*A.baumannii*	P.Salad	*aph-AI*	-	*anth-3*	-	-	*PmrA*
FD-99	d2	*A.baumannii*	Lettuce	-	-	*anth-3*	-	-	*PmrA*
FD-79	E	*A.baumannii*	C. meat	-	*aph-6*	*anth-3*	-	-	*PmrA*
CI-74	F	*A.baumannii*	Blood	*aph-AI*	-	-	-	-	*PmrA*
FD-58	G	*A.baumannii*	Veal	-	*aph-6*	*anth-3*	-	-	*PmrA*
FD-59	H	*A.baumannii*	Sucuk	-	*aph-6*	*anth-3*	-	-	*PmrA*
FD-72	I	*A.baumannii*	S.cheese	*aph-AI*	-	-	-	-	*PmrA*
CI-50	j1	*A.baumannii*	Catheter	-	-	-	-	-	*PmrA*
CI-51	j2	*A.baumannii*	Wound	-	-	-	-	-	*PmrA*
CI-72	j3	*A.baumannii*	Aspirate	-	-	*anth-3*	-	-	*PmrA*
CI-66	K	*A.baumannii*	Aspirate	-	-	-	-	-	*PmrA*
FD-100	l1	*A.baumannii*	Spinach	*aph-AI*	*aph-6*	-	-	-	*PmrA*
FD-101	l2	*A.baumannii*	P.cabbage	*aph-AI*	*aph-6*	-	-	-	*PmrA*
CI-49	m1	*A.baumannii*	Tissue	-	*aph-6*	-	-	-	*PmrA*
CI-51	m2	*A.baumannii*	Urine	-	-	-	-	-	*PmrA*
CI-73	n1	*A.baumannii*	Wound	*aph-AI*	-	-	-	-	*PmrA*
CI-15	n2	*A.baumannii*	Wound	*aph-AI*	-	-	-	-	*PmrA*
CI-2	O	*A.baumannii*	Urine	-	-	-	-	-	-
CI-47	p1	*A.baumannii*	Aspirate	*aph-AI*	-	*anth-3*	-	-	*PmrA*
CI-28	p2	*A.baumannii*	Sputum	-	-	-	-	-	-
CI-84	R	*A.baumannii*	Wound	-	-	-	-	-	*PmrA*
CI-60	s1	*A.baumannii*	Wound	-	-	-	-	*aadB*	*PmrA*
CI-64	s1	*A.baumannii*	C. fluid	-	-	*anth-3*	-	-	*PmrA*
CI-42	s2	*A.baumannii*	Wound	-	*aph-6*	-	-	*aadB*	*PmrA*
FD-56	t1	*A.baumannii*	Lettuce	-	*aph-6*	*anth-3*	-	-	*PmrA*
FD-80	t2	*A.baumannii*	Lettuce	*aph-AI*	*aph-6*	*anth-3*	*aadAI*	-	*PmrA*
CI-70	U	*A.baumannii*	Urine	-	-	-	-	-	*PmrA*
CI-14	V	*A.baumannii*	Blood	*aph-AI*	-	*anth-3*	-	-	*PmrA*
CI-41	Y	*A.baumannii*	Wound	-	*aph-6*	-	-	-	*PmrA*
CI-80	Z	*A.baumannii*	Aspirate	-	*aph-6*	-	-	-	*PmrA*
FD-62	X	*A.baumannii*	T.cheese	*aph-AI*	*aph-6*	-	*aadAI*	-	*PmrA*

Figure 2. PFGE dendrograms among the 34 strains of *A. baumannii* from human infection agents (20) and foods (14) are included. The closely-related clusters (≥80% similarity) are presented boxed.
FD: Food isolate, CI: Clinical Isolate, S. Cheese: String Cheese, P. salad: Packed Salad, C. Meat: Chicken Meat, P. Cabbage: Purple Cabbage, C. Fluid: Cerebrospinal Fluid, T. Cheese: Traditional Cheese.

Discussion and Conclusion

Due to its gaining of multidrug resistance pattern, *A. baumanni* became an important foodborne and nosocomial opportunistic pathogen (15, 21). The most abundant species of our foodborne (n=14, 51.9%) and, clinical strains were *A. baumanni* (n=50, 100%), *A. pitti* (n=5, 18.5%). These species and *A. calcoaceticus* (n=1, 3.7%) were also considered the *A. baumannii* group most constantly associated with nosocomial infections worldwide (26, 38, 39). In the current study, the rate of this group was 90.9% (70/77). Fewer phenotypic- resistant strains in food samples were found in our study. Only one *A. dijkshoorniae* strain was found MDR and this result (3.7%, MDR) was lower than previous reports in Portugal (29.8%, MDR in fruits and vegetables) and Iran (50%, MDR in chicken meat), respectively (5, 3). Overall, 92% of clinical isolates were resistant to at least five classes of antibiotics, hence meeting the criteria for extensive drug resistance (XDR resistance) (16). The frequency rate of antimicrobial resistance of *A. baumannii* recovered from clinical specimens was found between 88% and 94% for all tested antibiotics (Table 2). Similarly, in China, Pakistan, and Iran profiles with higher resistance (100%) were reported (15, 31, 40).

Screening for genes encoding AMEs demonstrated that 92% of the clinical isolates that are amikacin resistant contained the phosphotransferase gene *aphA6* with the rate of 22% (11/50). Other genes encoding AMEs included the adenylyltransferase genes *aadA1* 2% (1/50) and, *aadB* 4% (2/50) genes were found in this study (Table 3). Our results were lower than Mortazavi et al. (25)'s study in Iran. They reported as *aphA6*, *aadA1*, and *aadB* genes with the rate of 22.5%, 11.25%, and 30% respectively. They also found a positive correlation between *aadB* and *aphA6* genes positivity with high resistance against gentamicin and amikacin inconsistent with our research (Table 4). A similar study conducted in Germany by Wareth et al. (36) reported that 19% of strains were found resistant to amikacin and the new subclass of intrinsic aminoglycoside nucleotidyltransferase, *ant(3")-IIa*, was widely distributed in humans, animals, and milk powder samples. Subsequently, the intrinsic aminoglycoside nucleotidyltransferases (*aadA* and *aadAI* genes) were detected in 9 percent of the isolates. By contrast in our study, the amikacin resistance of our clinical strains was higher than their result (92%), and the *aph-AI* gene was the most prevalent in foodborne and clinical strains (38, 54.3%). Besides, the *anth(3'')-I* gene was widely seen in foodborne strains (19, 70%) and *aadA1* gene prevalence was less in our study (Table 3). The *aph(3'')-I* gene (70%) rate in clinical strains were higher than Moniri et al. (24)'s results (41.7%) in Iran, but lower than Wen et al. (37)'s results (85%) in China. Tested AME genes were more frequent among foodborne isolates than clinical strains and carried more multi- AME genes (P<0.001) (Table 3). Many of these genes are widespread in *Pseudomonas aeruginosa* and *A. baumannii* and mirror those described in a collection of MDR *A. baumannii* isolates from Europe (clone types I, II, and III) (16). In *Acinetobacter* spp.; some important AME genes can be located in plasmids [*ant(3'')-Ia, aadA1, aph(3')-VIa, aph(6)-Id*)], integron [*(ant(3'')-Ia, aadA1*], transposon [*ant(3'')-Ia, aadA1, aph(3')*], integrative conjugative element [*aph(6)-Id, aph(3'')-Ib*], chromosome [*aph(3'')*], and chromosomal genomic island [*aph(6)-Id*]. AME genes can be transferred by means of mobilizable or conjugative plasmids, natural transformation, or transduction (14, 22, 28). Thus, the findings of AME genes in our foodborne isolates mean that tested AME genes can be transferred by other pathogenic bacteria in food production processes (the use of contaminated/sewage water in the agricultural sector, unhygienic practices in slaughter and milk production processes (improper heating/pasteurization or contamination by food workers, etc.) or some of them carried in their chromosome/chromosomal genomic island.

In this study, 14% of clinical *A. baumannii* isolates were found colistin-resistant. This rate is higher than the resistance ratio (2.9%) reported from Southwestern Iran by Khoshnood et al. (18), and higher than previous works in Saudi Arabia and Pakistan, no colistin-resistant *A. baumannii* isolates were found in clinical samples (18, 30). These varying rates of resistance may arise from differences in the epidemiology and the infection treatment regulatory policies of respective countries, management patterns, and antibiotic use. The *pmrA* gene rate in clinical colistin-resistant and sensitive *A. baumannii* isolates was found (46, 92%) which were higher than Sepahvand et al. (30)'s study in Iran. In their study, they detected the *PmrA* gene at a rate of 70 percent and the *PmrB* gene at a rate of 30 percent. They reported that among the *A. baumanni* isolates carrying these genes, there are also colistin-resistant and sensitive ones. In our study, similar to the results of Sepahvand et al. (30)'s, colistin-resistant and susceptible *Acinetobacter* spp. strains carried the *PmrA* gene. The increased expression of the *PmrAB* system is necessary for *A. baumannii* resistance to colistin and the expression rate of *pmrA/pmrB* genes should be compared to colistin-sensitive strains (1). All foodborne strains carried *PmrA* genes but their sensitivity to colistin may be due to the chromosomes carried in this gene and also we did not measure the expression rate of the *PmrA*/*PmrB* genes (Figure 1).

The *aadA1* gene confers streptomycin and spectinomycin resistance, *aadB* gene confers tobramycin, gentamicin, and kanamycin resistance, *aphA6* gene confers amikacin, gentamicin, kanamycin, and neomycin

resistance (13). In clinical *A. baumannii* strains; there was a significant association between the harboring of *aph-AI* gene positivity and trimethoprim-sulfamethoxazole and gentamycin resistance (P=0.023; P=0.010); and *anth(3'')-I* gene positivity and colistin resistance (P=0.048); *PmrA* gene positivity and piperacillin-tazobactam, ceftazidime, meropenem, amikacin, and imipenem resistances (P=0.014; P=0.014; P=0.028, P=0.028; and P=0.014) were found respectively (Table 4). In the foodborne *Acinetobacter* spp. (Table 5) section of the results, a positive correlation was found between *aadAI* gene positivity and trimethoprim-sulfamethoxazole resistance (P=0.030). By this correlation, we can assume that in foodborne strains, trimethoprim-sulfamethoxazole resistance acquisition may be the result of the effect of the *aadAI* gene. The finding is in agreement with the fact that streptomycin and spectinomycin are the usual substrates for *aadAI* gene, but the correlation between trimethoprim-sulfamethoxazole and *aadAI* gene in foodborne strains means that a combination with streptomycin and spectinomycin usage (in agriculture/animal husbandry) may have entailed this result. A similar result was seen in our clinical strains (*aph-AI* gene positivity and trimethoprim-sulfamethoxazole and gentamycin resistance). However, other correlations were the unexpected substrates related to the genes; [*anth(3'')-I* and *PmrA* genes] which were in agreement with Sheikhalizadeh et al. (32)'s report in Iran [*ant(2')-Ia,aac(3')-IIa* encoding genes and related antibiotic non-susceptibility], and South Africa on clinical SAK strain of *A. baumannii* (9). These results may vary according to antibiotic combination usage in clinical settings. To this very day, we have not encountered any specialist text investigating the relationship between antibiotic resistance and aminoglycoside (AME) and colistin (*PmrA*) resistance genes in foodborne *Acinetobacter* spp. strains. Therefore, we were not able to make a comparison with a relevant field study.

The clonal relationships were not found between foodborne and clinical strains by PFGE. This difference in results may be due to various effectors including time, place, and methodology. For instance; we collected the samples during the Covid-19 pandemic. Therefore, we couldn't collect colonization strains from healthy patients. If we could have collected these isolates, they would have enabled us to reach wider data.

Interestingly, some closely related clusters have the same AME and *PmrA* genes; in the group of ''d'' and ''m''. Except for *aph-AI* and *aph-6*, all tested genes are shown in the same cluster. Similarly, in the group of ''j'', except for *anth(3'')-I*, all tested genes are shown in the same cluster; in the groups of ''l'' and ''n'' all tested genes carriage are shown in the same cluster (Figure 2). Thus, the same clones dissemination occured in foodborne and clinical strains. Our results are consistent with the literature; the cluster analysis showed that AME genes and *16S rRNA* methylase genes are often associated with genetic markers of moveable genetic elements (i.e., these genes are removable rear-mediated genetic elements). This acquired resistance mechanism facilitates horizontal spread (20).

Finally, *Acinetobacter* spp. from food can carry AME and *PmrA* genes and may rarely be resistant to aminoglycoside and carbapenems. However, the *A. baumanni* and *A. pitti* bacteria isolated from foods (especially from fruit and vegetables, chicken, turkey, and veal meat) have AME resistance genes at various rates and are more common than the clinical isolates. Foodborne *A. baumannii*, *A. pittii*, and *A. dijkshoorniae* strains may have been potential sources of the dissemination of AME and, *PmrA* genes confer to aminoglycoside and colistin resistance. The positive correlation between the positivity of *aph-AI*, *anth(3'')-I*, and *PmrA* genes in clinical *A. baumannii* isolates and resistance to various antibiotics are crucial findings. The spread of AME genes/resistance formation due to the positive relationship between *aadAI* gene positivity and trimethoprim-sulfamethoxazole resistance in foodborne isolates points to the food chain being a factor in the spread/formation of AME genes/resistance. Foodborne *A. baumanni* isolates are not genetically related to clinical strains which suggests that foodborne strains don't play a role in infection development. Our study has some limitations; low numbers of *Acinetobacter* spp. are analyzed in the study at hand. The cause of limitation is due to the difficulty of isolation of food and the difficulties encountered in the identification. Further studies including more *Acinetobacter* spp. isolates of foodborne and clinical with more antibiotics resistance, AME, *16SrRNA* methylase genes, as well as efflux pumps genes in wider geographical areas/countries are needed to ensure food safety in the food industry and lend to clinical microbiology.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

MT, ZE, and FK conceived and planned the experiments. MT carried out the experiments. MT, ZE, and FK planned and carried out the simulations. MT, ZE, and FK contributed to sample preparation. MT, ZE, and FK contributed to the interpretation of the results. MT took the

lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was carried out after the clinical samples were approved by Çukurova University Local Ethics Committee (Decision number: 14.06.2019-89).

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