The Effects of Antidepressants Fluoxetine, Sertraline, and Amitriptyline on the Development of Antibiotic Resistance in *Acinetobacter Baumannii*

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Abstract

In this study, we investigated the effects of antidepressants fluoxetine, sertraline, and amitriptyline on the development of antibiotic resistance in *Acinetobacter baumannii*. Susceptible clinical *A. baumannii* isolates were exposed to fluoxetine, sertraline, amitriptyline for 30 days, respectively. After exposure, the bacteria that developed resistance to gentamicin, imipenem, colistin, and ciprofloxacin were isolated and expression levels of some antibiotic resistance genes were compared with test bacteria in initial cultures using the quantitative Reverse-Transcriptase PCR method. The data obtained were analyzed using Student's t-test method. Increases in the MIC values of test bacteria were also determined after the exposure. The number of test bacteria that developed resistance and the MIC values of some bacteria were increased with the extension of exposure time. After exposure to fluoxetine and sertraline, decreases were observed for efflux and outer membrane porin genes in isolates that developed colistin resistance, and increases were observed in isolates that developed ciprofloxacin resistance. These observations suggest that these antidepressants have similar effects on the development of resistance. While the exposure to fluoxetine didn't result in the development of resistance to imipenem, it was observed after exposure to sertraline and amitriptyline, and a common decrease in *ompA* gene expression was determined in these isolates. This study is one of the preliminary investigations that demonstrates the role of non-antibiotic drugs on the development of antibiotic resistance. To the best of our knowledge, our findings report the comparative effects of selected antidepressants on the development of antibiotic resistance in *A. baumanni* for the first time in the literature.

Introduction

Antibiotics are an essential and versatile medication due to their uses in cases such as surgical procedures, organ transplantations, and the treatment of cancer patients (Munita and Arias 2016). Unnecessary and misuse of antibiotics in animals, the food sector, and agriculture fields as well as among the general public leads to the development of antibiotic resistance (Landers et al. 2012). Infections caused by resistant bacteria are difficult to treat, cause worse clinical outcomes and increase the risk of death, especially in intensive care units and patients with weak immune systems [Morehead and Scarbrough 2018; WHO 2020a). *Acinetobacter baumanii* is considered one of the antibiotic resistance threats in the United States. Despite the preventive actions taken on a global scale to tackle the consumption of antibiotics, resistance cannot be controlled. More holistic approaches are required to solve this problem (CDC 2019).

Depression affects more than 264 million people worldwide and negatively impacts on a person's life (WHO 2020b). Antidepressants are used to treat the symptoms of depressive disorders by correcting the chemical imbalances of neurotransmitters in the central nervous system (Alvano and Zeiher 2020). Fluoxetine and sertraline are grouped in the selective serotonin reuptake inhibitors (SSRIs), which are the most frequently prescribed drugs in the treatment of depression due to their low toxicity and limited side effects (Do et al. 2017). Fluoxetine is commonly used for various psychopathological states such as mood and eating disorders, obsessive-compulsive disorders, depression, and dysthymia in elderly people. Since it

has a long half-life and active metabolites, it has a different pharmacokinetic profile compared to other drugs in the SSRIs. A significant portion of the drug is excreted in the urine, while some of it is excreted in the faeces (Altamura et al. 1994). The fact that fluoxetine is excreted in the urine leads to its accumulation of certain amounts in wastewater, rivers, and groundwater. Hence, attention has been focused on the toxicity and ecotoxicity of fluoxetine (Brooks et al. 2003; Jin et al. 2018). The other commonly used SSRI in the treatment of depressive disorders is sertraline. Although it is mostly metabolized in the liver, less than 0.2% of the drug is excreted as is in the urine (Wang et al. 2001). Even though sertraline has been reported to have negative effects on algae, its effect on microbial communities has not yet been elucidated (Johnson et al. 2007; Yang et al. 2019). Amitriptyline classified in tricyclic antidepressants is used in the treatment of depression, nocturnal urination, neuropathic pain, panic disorder, attention-deficit/hyperactivity disorder, and obsessive-compulsive disorder (Şahin et al. 2008). Although amitriptyline is metabolized in the liver to its active metabolites, approximately 1% of the initial dose is excreted unchanged in the urine (Mastrianni et al. 2016).

It should be noted that the long-term usage of non-antibiotic drugs may affect both the microflora members and environmental microorganisms due to their improper disposal and accumulation into wastewater, respectively. Antidepressants are among these drugs (Jin et al. 2018). The aim of this study is to investigate the effects of antidepressants fluoxetine, sertraline, and amitriptyline on the development of antibiotic resistance in *A. baumannii*. This study is one of the primary investigations that demonstrates the role of non-antibiotic drugs in antibiotic resistance development.

Materials And Methods

Bacterial strains and growth conditions

Ten (gentamicin, imipenem, colistin, ciprofloxacin) susceptible and one resistant clinical *A. baumannii* isolates were used. *A. baumannii* ATCC 1709 (susceptible strain) and *A. baumannii* ATCC 1799 (resistant strain) were used as standard strains. Antibiotic susceptibilities of all test bacteria were confirmed by using the disc diffusion and minimum inhibitory concentration (MIC) tests (EUCAST 2018). In susceptibility tests, *Escherichia coli* ATCC 25922 strain was used as the control.

Trypticase Soy Broth (Merck) and Trypticase Soy Agar (Merck) were used as initial growth media. Mueller Hinton Agar (MHA) (Merck) and Mueller Hinton Broth (MHB) (Merck) were used in antibiotic susceptibility tests. The antidepressant exposure step was carried out in Lysogeny Broth (LB) (Merck). MHA plates with 4 μg ml^{-1} gentamicin (Sigma), 8 μg ml^{-1} imipenem (Sigma), 2 μg ml^{-1} colistin (Sigma), and 1 μg ml^{-1} ciprofloxacin (Sigma) were used to isolate antibiotic-resistant test bacteria. All test bacteria were incubated at 37°C for 18-24 h.

Detection of ade efflux pump and outer membrane porin genes

The presence of ade efflux pump genes *adeA*, *adeB*, *adeC*, *adeR*, *adeS*, and outer membrane porin genes *ompA*, *ompW* in test bacteria were demonstrated by Polymerase Chain Reaction (PCR) method by using

primers described in Table 1. Gel electrophoresis was used for the detection of the amplification products visualized with SafeView Classic (ABM, Canada). The size of PCR products was compared with a 100bp DNA Ladder (New England BioLabs, ABD) (Lopes 2012; McConnell et al. 2012; Jassim et al. 2016; Catel-Ferreira et al. 2016; Sepahvand et al. 2017).

Table 1
The sequences of primers used for the polymerase chain reaction to detect *Acinetobacter baumannii* efflux pump and outer membrane porin genes

Gene	Primer	Sequence	Reference
adeA	F	5'-ATCTTCCTGCACGTGTACAT-3'	(Jassim et al. 2016)
	R	5'-GGCGTTCATACTCACTAACC-3'	
adeB	F	5'-GTATGAATTGATGCTGC-3'	(Jassim et al. 2016)
	R	5'-CACTCGTAGCCAATACC-3'	
adeC	F	5'-AGCCTGCAATTACATCTCAT-3'	(Jassim et al. 2016)
	R	5'-TGGCACTTCACTATCAATAC-3'	
adeS	F	5'-TGCCGCCAAATTCTTTATTC-3'	(Lopes 2012)
	R	5'-TTAGTCACGGCGACCTCTCT-3'	
adeR	F	5'-CGCTCTAGTGCATCGCTATC-3'	(Lopes 2012)
	R	5'-GCATTACGCATAGGTGCAGA-3'	
ompA	F	5'-TCTTGGTGGTCACTTGAAGC-3'	(McConnell et al. 2012)
	R	5'-ACTCTTGTGGTTGTGGAGCA-3'	
ompW	F	5'-TATGGATCCGGTAATTGGCAAGTAAAATTTGGG3'	(Catel-Ferreira et al. 2016)
	R	5'-TATAAGCTTTTAGAATTTATAGCTATAGCC-3'	
16SrDNA	F	5'-TCAGCTCGTGTCGTGAGATG-3'	(Sepahvand et al. 2017)
	R	5'-CGTAAGGGCCATGATG-3'	

Determination Of Minimum Inhibitory Concentration Values

Before and after exposure to fluoxetine, sertraline, and amitriptyline, MIC values of test bacteria against gentamicin, imipenem, colistin, and ciprofloxacin were determined by the microdilution method (EUCAST 2018). Increase in the MIC values after exposure was detected. Each test bacterium was tested in triplicate.

Fluoxetine, sertraline, and amitriptyline exposure and isolation of resistant bacteria against test antibiotics

Initially, exposure concentrations of fluoxetine, sertraline, and amitriptyline were determined by MIC test (CLSI 2009). 40 µl suspension of test bacteria (adjusted to 10^8-10^9 cfu/ml) was added into 3.96 ml LB containing the various concentrations of antidepressants, fluoxetine hydrochloride (Sigma) (2.5-5-10 µg ml^{-1}), sertraline hydrochloride (Sigma) (1.25-2.5-5 µg ml^{-1}) and amitriptyline hydrochloride (Sigma) (2.5-5-10 µg ml^{-1}). After incubation at 37°C for 18-24 h, 40 µL of the test bacteria cultures were transferred into 3.96 mL fresh LB containing the above-stated concentrations of fluoxetine, sertraline, and amitriptyline and incubated again. This subculturing procedure was repeated for 30 days incessantly. On the 5th, 10th, and 30th days of this procedure, 100 µl of exposed test bacteria samples were plated on the MHA plates with 4 µg ml^{-1} gentamicin, 8 µg ml^{-1} imipenem, 2 µg ml^{-1} colistin, and 1 µg ml^{-1} ciprofloxacin. After 24 hours of incubation at 37°C, the colonies formed on the MHA containing test antibiotics were considered as resistant. Test bacteria that were subcultured in LB broth without antidepressants were used as the control group (Jin et al. 2018).

Quantitative Reverse-transcriptase Pcr Analysis Of Target Genes

Expression levels of target genes of test bacteria were determined by Quantitative Reverse-Transcriptase PCR (RT-qPCR). Pre-exposure and post-exposure expression levels of the ade efflux pump genes and outer membrane porin genes were compared (Eleaume and Jabbouri 2004; Kart et al. 2020). The total RNA was extracted using an RNA Isolation Kit (RNeasy Mini Kit, Hilden, Qiagen) according to the manufacturer's instructions. The total RNA was quantified in each sample using a NanoDrop spectrophotometer (Qiagen). cDNA was synthesized by NucleoGene RNA to cDNA Mix Kit (NucleoGene, Turkey) according to the manufacturer's instructions. A NucleoGene qPCRSYBR Green Master Mix Kit (2x) (NucleoGene, Turkey) was used for the determination of expression levels of the target genes. The RT-qPCR analysis was performed for each 20 µl PCR reaction mixture (10 µl NucleoGene qPCR Sybr Green Master Mix, 6.5 µl ddH_2O, 2.5 µl cDNA template, 0.5 µl Forward Primer and 0.5 µl Reverse Primer) using the primers indicated in Table 1. Relative gene expression levels were calculated with the $2^{-\Delta\Delta CT}$ method using an RT2 Profiler PCR Array Data Analysis v3.5 (Qiagen, Hilden) analysis program. All data were normalized for 16S rRNA housekeeping gene levels.

Statistical analysis

Statistical data analysis was performed using the SPSS programme (Version 23, SPSS, Chicago, IL, USA). Student's t-test was used for comparing the pre-and post-exposure expression levels of the target genes. P values <0,05 were considered significant.

Results And Discussion

Detection of ade efflux pump and outer membrane porin genes

Agarose gel electrophoresis images of ade efflux pump and outer membrane porin genes are given in Figure 1.

Mic Values Of Fluoxetine, Sertraline, And Amitriptyline

MIC values of fluoxetine, sertraline, and amitriptyline are given in Table 2. According to these values, exposure concentrations were determined as 2.5-5-10 μg ml^{-1} for fluoxetine hydrochloride and amitriptyline hydrochloride, 1.25-2.5-5 μg ml^{-1} for sertraline hydrochloride.

Table 2
MIC values of test bacteria against fluoxetine, sertraline, amitriptyline (25-0.195 μg ml^{-1}). (**1-10**: Susceptible isolates, **11**: Resistant isolate, **12**: *A. baumannii* ATCC 1709, **13**: *A. baumannii* ATCC 1799)

	Fluoxetine	Sertraline	Amitriptyline
1	25	12.5	-
2	25	12.5	-
3	12.5	12.5	-
4	12.5	12.5	-
5	25	12.5	-
6	12.5	12.5	-
7	12.5	12.5	-
8	6.25	6.25	25
9	25	12.5	-
10	12.5	12.5	-
11	25	12.5	-
12	12.5	12.5	25
13	25	25	-

Antibiotic Resistance Development After Exposure

The number of isolates that developed phenotypic resistance to gentamicin, imipenem, colistin, and ciprofloxacin on the 5th, 10th, and 30th days of the exposure are given in Figure 2. Resistant isolates are indicated in Table 3.

Table 3
Susceptible test bacteria developed phenotypic resistance on the exposure times

EXP day	Test ABX	Fluoxetine (µg ml^{-1})			Sertraline (µg ml^{-1})			Amitriptyline (µg ml^{-1})			CTRL
		2,5	5	10	1.25	2,5	5	2,5	5	10	
5th	**CIP**	12	7, 8	4	-	-	4, 7, 12	-	-	4, 7, 10	8
	GEN	4	-	4, 5, 6	4	-	5, 6, 7	-	1, 2	4, 10	-
	IPM	-	-	-	-	-	7	-	-	10	-
	CST	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10
10th	**CIP**	12	7, 8	4	-	-	4, 7, 12	-	-	3, 4, 7, 10	8
	GEN	3, 4	3, 7	4, 5, 6	4	4	5, 6, 7	4	1, 2	4, 10	-
	IPM	-	-	-	-	-	7	-	-	10	-
	CST	1-10	1-10	1-10	1-10, 12	1-10, 12	1-10	1-10, 12	1-10, 12	1-10	1-10, 12
30th	**CIP**	12	4, 5, 7, 8, 10	4	7	4, 5, 6, 7	1, 4, 7, 12	1, 2, 6, 12	1, 2, 5, 6, 12	3, 4, 6, 7, 10	4, 7, 8, 9
	GEN	3, 4, 12	3, 7, 12	3, 4, 5, 6, 8,10, 12	4, 5, 6	4, 6	5, 6, 7	2, 3, 4, 5, 6, 9, 12	1, 2, 4, 5, 7, 8	2, 4, 10	7, 8, 9
	IPM	-	-	-	-	-	7	-	-	10	8
	CST	1-10, 12	1-10	1-10	1-10, 12	1-10, 12	1-10	1-10, 12	1-10, 12	1-10, 12	1-10, 12

EXP day: Exposure Day, **Test ABX**: Test antibiotics, **CTRL**: Susceptible test bacteria that subcultured in LB broth without any antidepressant for 30 days, Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IPM), Colistin (CST), **1-10**: Susceptible isolates, **12**: *A. baumannii* ATCC 1709.

Considering the results of phenotypic resistance development after exposure, it was observed that exposure to fluoxetine did not cause imipenem resistance. However, at the end of the fluoxetine exposure of the 30th day, an increase was observed in the number of isolates that developed resistance to ciprofloxacin and gentamicin. It was observed that exposure to the highest concentrations of sertraline and amitriptyline caused imipenem resistance in one isolate in each group at the end of the 5th day. In addition, at the end of the 30th day, imipenem resistance was observed in one of the isolates in the control group. Amitriptyline exposure caused the development of gentamicin resistance in all test bacteria, at the end of the 30th day. In addition, it was also determined that three isolates (7, 8, and 9) in the control group developed resistance to gentamicin. The resistance to ciprofloxacin and gentamicin largely developed after exposure to amitriptyline. Since colistin resistance was seen in all isolates in the control group, it could not be correlated with the resistance developed after antidepressant exposure.

Jin et al. (2018) were the first to report that exposure to fluoxetine induces antibiotic resistance in *E. coli*. In this study, as a result of 10 hr exposure of *E. coli* to fluoxetine, the expression levels of multidrug efflux pump genes *acrB* and *acrD* increased, while the expression levels of the outer membrane porin genes *ompF* and *ompW* decreased. The bacterium protected itself from exposure to fluoxetine by expelling the drug from the cell and preventing the entry of the drug into the cell. It was determined that exposure to fluoxetine for 30 days caused an increase in chloramphenicol, amoxicillin, and tetracycline resistance. Also, these mutants exhibited multiple resistances against fluoroquinolones, aminoglycosides, and beta-lactams.

Our study is important in terms of investigating the effects of sertraline and fluoxetine, as both antidepressants have the same mechanism of action and amitriptyline which acts with a different mechanism, on the development of antibiotic resistance in *A. baumannii* for the first time in the literature.

In order to observe the effect of antidepressants on resistance genes, gene expression values of some test bacteria that developed resistance to test antibiotics after exposure to fluoxetine, sertraline, and amitriptyline were compared relative to controls that were not exposed to any antidepressant (Table 4-6).

Table 4
Relative fold change of resistance gene expression levels of some test bacteria that develop resistance after exposure to amitriptyline

AMITRIPTYLINE	*adeA*	*adeB*	*adeC*	*adeR*	*adeS*	*ompA*	*ompW*
4 (CIP/5th day)	4.61	4.96	-	-2.55	5.64	0.54	0.75
3 (CIP/10th day)	-	-	1.02	1.195	7	*-0.66	4.85
6 (CIP/30th day)	0.94	3.16	1.33	2.35	12.32	2.34	15.02
7 (CIP/30th day)	20.27	19.71	-	19.27	28.43	1.61	8.34
12 (CIP/30th day)	-1.63	8.48	7.72	7.27	8.64	-10.60	16.93
10 (GEN/5th day)	0.03	-2.93	1.14	-4.45	5.12	7.41	9.82
4 (GEN/10th day)	-10.21	*-0.42	-12.89	4.00	-9.04	*0.41	3.01
12 (GEN/30th day)	-9.81	5.06	3.09	1.79	4.01	-15.23	1.61
9 (GEN/30th day)	12.45	12.77	-	12.25	-	-9.01	27.35
10 (IPM/5th day)	10.00	-7.74	-8.22	-4.67	17.15	-3.77	*0.07
7 (CST/5th day)	-2.37	-5.51	-	-7.31	13.75	3.20	-1.54
4 (CST/5th day)	-2.68	2.50	26.39	13.64	10.64	3.52	4.81
12 (CST/10th day)	19.07	4.41	*-0.78	6.44	-5.93	-7.31	-3.06

*Statistically insignificant (P≥0.05), Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IPM), Colistin (CST). All isolates were compared with their controls not exposed to amitriptyline.

Table 5
Relative fold change of resistance gene expression levels of some test bacteria that develop resistance after exposure to sertraline

SERTRALINE	*adeA*	*adeB*	*adeC*	*adeR*	*adeS*	*ompA*	*ompW*
12 (CIP/5th day)	23.19	3.37	12.16	5.13	-4.96	-1.09	-2.86
13 (CIP/5th day)	21.22	7.64	1.63	-2.85	-9.92	-5.59	-4.00
7 (CIP/5th day)	9.74	-7.58	3.36	-8.21	-20.45	-1.74	-0.93
4 (CIP/5th day)	-1.14	8.04	-	-5.62	2.97	-4.07	1.07
7 (CIP/10th day)	-14.35	-3.58	-7.97	-4.38	3.34	3.60	8.97
7 (CIP/30th day)	20.27	19.71	-	19.27	28.43	1.61	8.34
5 (GEN/5th day)	-4.44	-2.28	1.62	5.72	13.27	5.53	24.16
6 (GEN/5th day)	-34.74	-24.71	-8.66	17.70	-26.26	-7.05	-42.84
12 (GEN/5th day)	22.89	4.42	10.21	6.09	-4.52	-1.61	-1.23
4 (GEN/10thday)	-1.40	0.74	*-0.71	5.66	-19.46	*0.85	-4.41
5 (GEN/30th day)	-6.41	-2.54	-4.28	13.72	35.24	5.86	1.09
7 (IPM/5th day)	-8.49	9.84	10.87	-5.80	8.41	-5.56	7.58
7 (CST/5th day)	-3.20	-7.85	-	-10.88	-19.71	*-0.96	1.03
4 (CST/5th day)	-28.41	-26.35	-35.19	-14.69	-35.19	-5.52	-29.85
12 (CST/5th day)	-18.77	-3.55	-10.96	-5.85	*0.15	-3.48	-2.65

*Statistically insignificant (P≥0.05), Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IPM), Colistin (CST). All isolates were compared with their controls not exposed to sertraline.

Table 6
Relative fold change of resistance gene expression levels of some test bacteria that develop resistance after exposure to fluoxetine

FLUOXETINE	*adeA*	*adeB*	*adeC*	*adeR*	*adeS*	*ompA*	*ompW*
12 (CIP/5th day)	20.90	3.88	12.01	8.13	4.68	*-0.865	-1.85
7 (CIP/5th day)	7.60	-3.31	-6.49	-3.43	-20.40	*0.009	7.20
4 (CIP/5th day)	-2.73	5.25	-	-9.71	-1.05	*-0.375	-2.56
5 (CIP/30th day)	-3.85	1.00	-1.72	*-0.31	7.15	5.54	3.65
4 (CIP/30th day)	-	-	5.30	11.21	9.17	4.85	3.25
5 (GEN/5th day)	-6.00	-7.96	6.11	-6.80	1.88	-8.60	1.60
6 (GEN/5th day)	*-0.43	-1.99	-10.26	-6.35	-1.05	-1.55	3.46
7 (GEN/10th day)	1.04	2.27	-	15.96	10.34	8.32	7.24
11 (GEN/10th day)	-	-	0.40	-3.42	4.03	*0.44	-3.13
12 (GEN/30th day)	-11.51	4.33	15.94	11.97	3.23	6.51	2.18
7 (CST/5th day)	-1.13	-3.03	-	-5.75	-17.27	1.13	-2.23
4 (CST/5th day)	2.45	*-0.63	*-0.65	-5.60	*-0.65	2.09	-1.65

*Statistically insignificant (P≥0.05), Ciprofloxacin (CIP), Gentamicin (GEN), Colistin (CST). All isolates were compared with their controls not exposed to fluoxetine.

Decreases in the expression of all genes (except *ompA* and *ompW* for the number 12 strain) against ciprofloxacin were observed after exposure to amitriptyline, regardless of time. When all isolates with gentamicin resistance were evaluated together, the expression increase was determined only for the *adeC* and *ompW* genes. Much like the result obtained after exposure to sertraline, isolates that developed resistance to imipenem also found decreases in *adeR* and *ompA* expressions. For colistin-resistant isolates, a consistent result could not be obtained between the resistance genes tested and resistance development (Table 4).

An increase in expression of *adeR*, *adeS*, and *ompA*, and *ompW* was observed against ciprofloxacin after 30 days of exposure compared to 5 days after sertraline exposure. Regardless of the time, reductions in all genes against colistin were observed, while reductions in *adeR* and *ompW* genes were observed against imipenem. For isolates that developed resistance to gentamicin, a consistent result could not be obtained between tested resistance genes and resistance development (Table 5).

After exposure to fluoxetine, increased expression was observed in *adeR*, *adeS*, *ompA*, and *ompW* against ciprofloxacin, and *adeC*, *adeR*, *ompA*, and *ompW* against gentamicin, after 30-day exposure compared to 5-day exposure. Decreases in the *adeB*, *adeR*, *adeS*, and *ompW* genes against colistin were detected regardless of time (Table 6).

In our study, expression values of *A. baumannii* ATCC 1709 and *A. baumannii* ATCC 1799 that did not develop resistance after exposure for the tested genes were compared (Figure 3). In *A. baumannii* ATCC 1709, decreased expressions of *adeR*, *adeS*, and *ompA* for ciprofloxacin, *adeC* for gentamicin, and *adeB*, *adeC*, *adeS*, and *adeR* for imipenem were observed.

In this study, two isolates were selected for each antibiotic group from the isolates that developed resistance after exposure to different antidepressant concentrations for the 5th, 10th, and 30th days. In these isolates, mRNA expression levels of efflux pump genes *adeA, adeB, adeC, adeR, adeS*, and outer membrane porin protein genes *ompA* and *ompW* were measured using the RT-qPCR method. Expression data of isolates with resistance development were compared with control groups of the same isolate that were not exposed to antidepressants, thus the effects of resistance development on resistance genes were examined.

Although the mechanism of colistin resistance in *A. baumanii* is not known exactly, it is thought to occur as a result of modifications in the outer membrane lipopolysaccharides, resulting in decreased affinity of the antibiotic to its target site and decreased expression of *ompW*-like outer membrane porins (Dal et al. 2012). In our results, a decrease was detected in all genes of colistin-resistant isolates after sertraline exposure, and statistically significant decreases were observed in *adeB, adeR*, and *adeS* gene expressions, especially *ompW* against fluoxetine.

The AdeABC active drug pump system is responsible for the multi-drug resistance of *A. baumanii*, and their overexpression causes the development of resistance against many antibiotics (Wieczorek et al. 2008). *adeS*, one of the regulatory genes, activates the transcription of adeABC pump proteins by causing phosphorylation of *adeR* (Dal et al. 2012). The study of Lari et al. (2018) found a significant correlation between ciprofloxacin resistance and upregulated *adeB*. In our results, in line with the literature, increases in *adeR, adeS, ompA*, and *ompW* expressions were observed in isolates that developed resistance to ciprofloxacin after exposure to both fluoxetine and sertraline. The opposite results were obtained after exposure to amitriptyline, and reductions were observed in all genes (except for the 12th strain). This may be because amitriptyline has a different mechanism of action compared to the other two antidepressants. Amitriptyline and fluoxetine played a similar role in the development of gentamicin resistance, causing an increase in *adeC* and *ompW* resistance genes.

It is known that omp porin proteins found in the cell wall of *A. baumannii* prevent the entry of some molecules into the cell while preventing the exit of others from the cell, giving the cell wall selectivity. For this reason, the loss of these proteins in the outer membranes plays an important role in the emergence of antimicrobial resistance (Dal et al. 2012). While the exposure of our isolates to fluoxetine did not result in the development of resistance to imipenem, resistance to imipenem was observed after exposure to sertraline and amitriptyline, and a common decrease in the *ompA* gene expression was determined in these isolates.

Increase of the minimum inhibitory concentration values of test bacteria

Before and after exposure, MIC values of the bacteria against test antibiotics were given in Table 7. LB broth without any antidepressant was used as a control.

Table 7
Pre- and post-exposure MIC values of test bacteria against test antibiotics

	Exposure concentrations of antidepressants	Test ABX	Pre-exposure	Post-exposure
1	Amitriptyline 2,5 μg ml^{-1}	GEN	1	4
		CST	<0.25	>32
2	Fluoxetine 10 μg ml^{-1}	CST	<0.25	1
	Sertraline 1,25 μg ml^{-1}	IPM	<0.25	2
		CST	<0.25	2
	Amitriptyline 2,5 μg ml^{-1}	CST	<0.25	8
3	Fluoxetine 2,5 μg ml^{-1}	CST	<0.25	4
	Amitriptyline 10 μg ml^{-1}	CIP	<0.25	>32
		GEN	1	>32
		CST	<0.25	2
6	Sertraline 2,5 μg ml^{-1}	CIP	<0.25	1
7	Sertraline 5 μg ml^{-1}	CIP	<0.25	>32
		GEN	0.5	>32
		IPM	<0.25	32
8	Sertraline 2,5 μg ml^{-1}	GEN	0.5	1
	Sertraline 1,25 μg ml^{-1}	GEN	0.5	1
	Amitriptyline 10 μg ml^{-1}	GEN	0.5	1
	Amitriptyline 2,5 μg ml^{-1}	GEN	0.5	1
9	Fluoxetine 5 μg ml^{-1}	CST	<0.25	1
	Fluoxetine 2,5 μg ml^{-1}	CST	<0.25	4
	Sertraline 1,25 μg ml^{-1}	CST	<0.25	16
10	Fluoxetine 10 μg ml^{-1}	GEN	1	2
	Fluoxetine 2,5 μg ml^{-1}	CST	<0.25	8

Test ABX: Test antibiotics, Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IPM), Colistin (CST). (**1-10**: Susceptible isolates, **11**: Resistant isolate, **12**: *A. baumannii* ATCC 1709, **13**: *A. baumannii* ATCC 1799)

	Exposure concentrations of antidepressants	Test ABX	Pre-exposure	Post-exposure
	Amitriptyline 10 µg ml^{-1}	CIP	<0.25	>32
		GEN	1	>32
		IPM	<0.25	32
11	LB broth	IPM	4	32
	Amitriptyline 10 µg ml^{-1}	IPM	4	32
	Amitriptyline 5 µg ml^{-1}	IPM	4	16
	Amitriptyline 2,5 µg ml^{-1}	IPM	4	32
12	LB broth	GEN	<0.25	1
	Fluoxetine 2,5 µg ml^{-1}	CIP	<0.25	1
		CST	<0.25	2
	Sertraline 5 µg ml^{-1}	CST	<0.25	32
	Sertraline 2,5 µg ml^{-1}	GEN	<0.25	1
13	Sertraline 5 µg ml^{-1}	IPM	16	32
	Sertraline 1,25 µg ml^{-1}	CST	<0.25	1
	Amitriptyline 10 µg ml^{-1}	CST	<0.25	1

Test ABX: Test antibiotics, Ciprofloxacin (CIP), Gentamicin (GEN), Imipenem (IPM), Colistin (CST). (**1-10**: Susceptible isolates, **11**: Resistant isolate, **12**: *A. baumannii* ATCC 1709, **13**: *A. baumannii* ATCC 1799)

Considering the pre- and post-exposure MIC values of all test bacteria, it was observed that MIC values of isolates that developed imipenem resistance as a result of exposure to sertraline and amitriptyline increased >128-fold compared to pre-exposure. The MIC value of the isolate in the control group, which was observed to develop phenotypical resistance to imipenem, did not increase compared to the pre-exposure. It was observed that MIC values of some isolates that developed resistance to gentamicin because of exposure to amitriptyline increased at rates ranging from 2->32-fold compared to pre-exposure. Besides MIC value of isolate number 10, which developed resistance to gentamicin as a result of exposure to fluoxetine, increased 2-fold compared to the pre-exposure. MIC value of isolate 7, which developed resistance to gentamicin after sertraline exposure, increased >64-fold compared to pre-exposure. MIC values of some isolates that developed resistance to ciprofloxacin because of exposure to amitriptyline increased >128-fold compared to pre-exposure. After exposure to fluoxetine, it was observed that the MIC value of isolate 12, which developed resistance to ciprofloxacin, increased >4-foldcompared to the pre-exposure. It was observed that the MIC values of isolates 6 and 7, which developed resistance to ciprofloxacin because of sertraline exposure, increased by 2 and >128-fold, respectively, compared to pre-

exposure. Since colistin resistance was seen in all isolates in the control group, it could not be correlated with the resistance developed after antidepressant exposure. However, an increase in the MIC values of some isolates was observed. When all MIC values of test bacteria are considered, we can say that exposure to amitriptyline causes the highest increase in MIC values, and exposure to fluoxetine causes the lowest increase.

With the extension of exposure time to fluoxetine, sertraline, and amitriptyline, the number of test bacteria that developed antibiotic resistance and the MIC values of some bacteria were increased. After exposure to fluoxetine and sertraline, decreases were observed for efflux and outer membrane porin proteins in isolates that developed colistin resistance, and increases were observed in isolates that developed ciprofloxacin resistance, suggesting that these antidepressants have similar effects in the development of antibiotic resistance. However, the study of two isolates for each antibiotic group causes limitations in the general interpretation of the results. In future studies, it will be possible to reach a clearer conclusion by examining these genes for more isolates that develop resistance.

Declarations

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Conflict of Interest

The authors have no conflicts of interest relevant to this study to disclose.

Ethical Statements

Ethical approval was received from Ethics Committee of Ankara University Faculty of Medicine [Reference No: I5-195-19].

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Figures

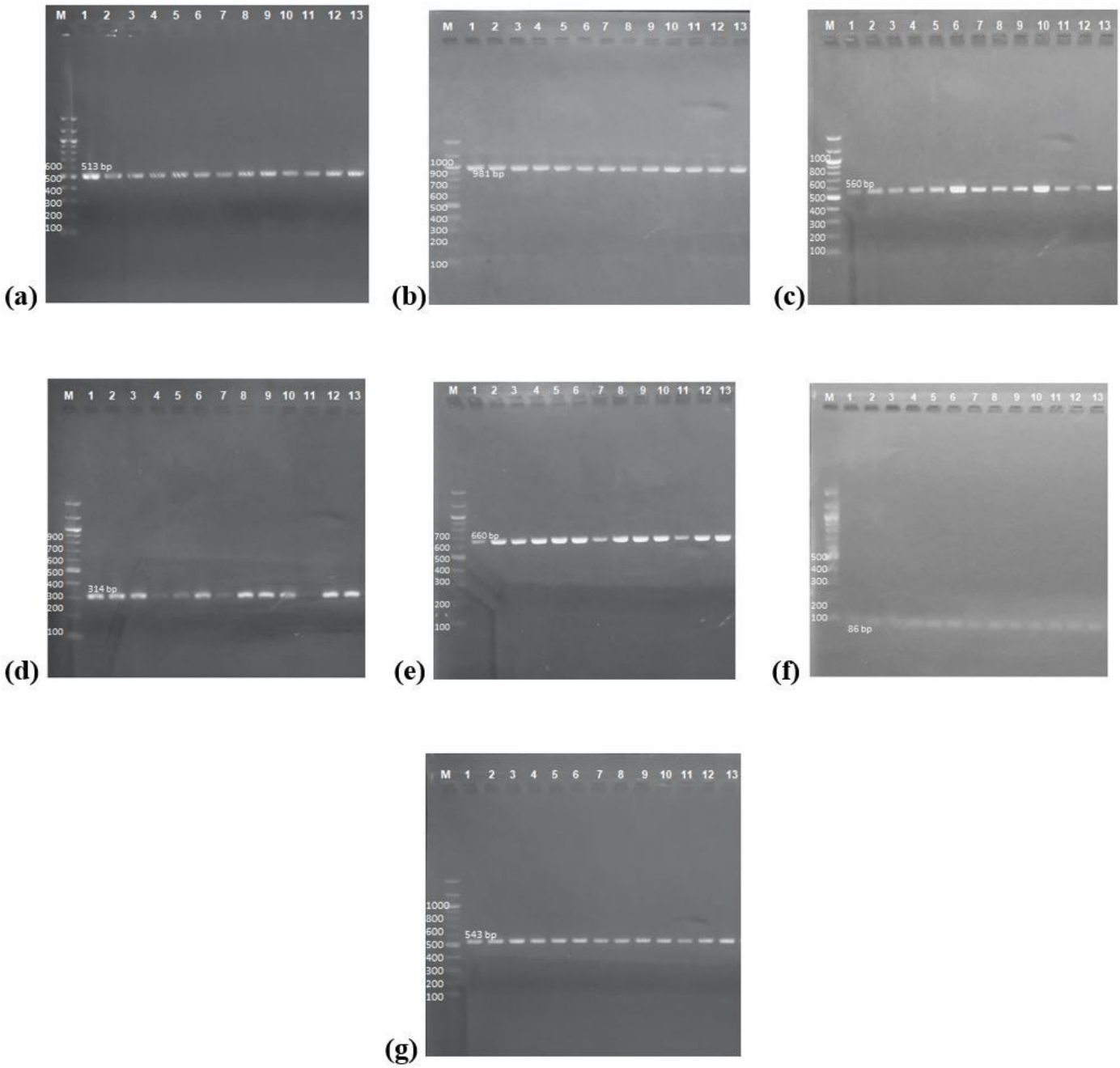

Figure 1

Agarose gel electrophoresis images of ade efflux pump and outer membrane porin genes (**M:** DNA Ladder (100 bp), **1-10:** Susceptible isolates, **11:** Resistant isolate, **12:** *A. baumannii* ATCC 1709, **13:** *A. baumannii* ATCC 1799) (a) *adeA* (513bp), (b) *adeB* (981 bp), (c) *adeC* (560 bp), (d) *adeR* (314bp), (e) *adeS* (660 bp), (f) *ompA* (86 bp), (g) *ompW* (543bp)

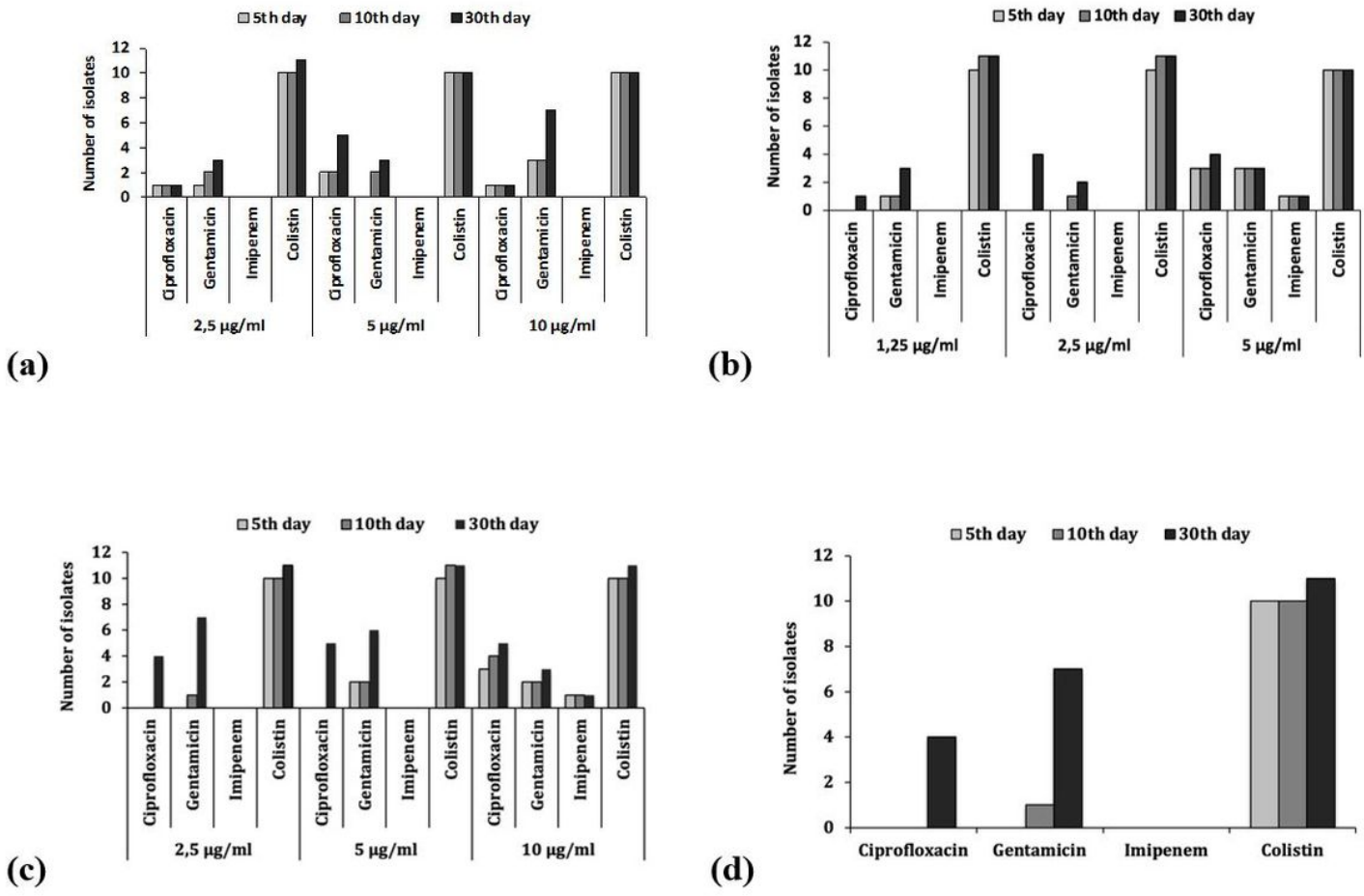

Figure 2

Phenotypic antibiotic resistance development of susceptible test bacteria on the 5th, 10th, and 30th days of the exposure. Susceptible clinical isolates and *A. baumannii* ATCC 1709 are the susceptible test bacteria. (a) Fluoxetine exposure, (b) Sertraline exposure, (c) Amitryptiline exposure, (d) Control (susceptible test bacteria subcultured in LB broth without any antidepressant)

Figure 3

Comparison of gene expression values of *A. baumannii* ATCC 1709 and *A. baumannii* ATCC 1799 exposed to antidepressants