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Research Article

Antimicrobial Susceptibility Patterns of *Bacillus anthracis* Isolates Obtained from Different Origins

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Abstract

In this retrospective study, it was evaluated the antimicrobial susceptibility profiles of *Bacillus anthracis* isolates obtained from human, various samples (blood, spleen, lung, liver, meat) of dead animals (cattle, sheep, dog, horse) that died from anthrax and soil samples from the animal burial areas between 2012 and 2023 from Kars province of Türkiye. In this context, a total of 87 *B. anthracis* isolates obtained from 1 human, two horses, 51 cattle, 7 sheep, 1 dog, and 25 soil were investigated. The isolates were confirmed as *B. anthracis* using protective antigen *(PA)* and capsule *(Cap)* gen specific PCRs. The Kirby-Bauer disk diffusion method was used for deternination of antimicrobial susceptibility. Ten antimicrobials including penicillin, amoxicillin, trimethoprim-sulfometoxazole, erythromycin, meropenem, streptomycin, ofloxacin, ciprofloxacin, chloramphenicol, and clindamycin were tested. As a result of PCR, all isolates were confirmed as fully virulent field strains of *B. anthracis*. All isolates were found as susceptible to penicillin, amoxicillin, ofloxacin and ciprofloxacin. Since the last studies in the region, a change in the antimicrobial profile of *B. anthracis* strains was observed only for trimethoprim-sulfomethoxazole among the antimicrobials tested, a transition from susceptibility to resistance. In conclusion, penicillin and amoxicillin, are still the antibiotic of first choice for the prophylaxis and treatment of anthrax. Ofloxacin and ciprofloxacin are also effective enough to be prescribed for treatment.

Keywords: Animal, Antimicrobial susceptibility, *Bacillus anthracis,* Human, PCR, Soil

Introduction

Anthrax is a sporadic infection of many warm-blooded animals (camels, horses, cats and dogs, etc.) in particular of herbivores (cattle and sheep) [1]. Although this disease, which can occasionally be transmitted from infected animals to humans by direct or indirect routes, is becoming less common in the world and our country, it continues its zoonotic existence and continues to be a global threat associated with bioterrorism as a biological weapon in both developed and developing countries [2].

The agent of anthrax is *Bacillus anthracis*. The agent is a pathogen that is Gram-positive, immobile, encapsulated, spore forming, and can grow as aerobic or facultative anaerobic [3,4]. Spores of *B. anthracis* are highly resistant to unfavourable environmental conditions. Spores remain viable for many years in contaminated environments and constitute an important source of infection in grazing animals for long term [5]. The expression of the pathogenic activity of *B. anthracis* in animals is mediated by the capsule localised on the pXO2 plasmid, which confers antiphagocytic properties, and a complex of three toxic proteins: protective antigen (PA), edema factor (EF), and lethal factor (LF) localised on the pXO1 plasmid [6,7].

In regions where anthrax is endemic, contact with infected

livestock or animal products during domestic slaughter, disposal of butchery waste, processing and disposal of cattle carcasses are the main sources of human infections; these are the primary factors that put livestock keepers and farmers at high risk of infection [8,9]. The severity of disease in humans depends on the patient's natural and adaptive immunity, the virulence of the agent and the number of times it enters the body [10]. Anthrax occurs in humans in different forms such as cutaneous, injection, gastrointestinal or inhalation, and cutaneous anthrax accounts for approximately 95% of infections [3]. Sepsis and meningoencephalitis are rare complications resulting from the spread of primary lesions [3,11]. The incidence of other infection types has been reported for inhalation anthrax, gastrointestinal anthrax and primary meningitis as 12% 5% 4%, respectively [12,13].

In the treatment of anthrax, the use of antibiotics is vital in line with the diagnosis [11,14,15]. The range of antibiotics to which *B. anthracis* is susceptible *in vitro* is quite wide and clinical isolates are sensitive to various antibiotics such as penicillin, carbapenems, aminoglycosides, macrolides, quinolones, vancomycin, rifampicin, tetracyclines, clindamycin, cefazolin, and linezolid [9,11]. The region and the severity of the disease influence the application of different antibiotic treatment strategies in anthrax [9,16]. Therefore, the first-line drugs for naturally occurring cases of anthrax are penicillin G and amoxicillin. Especially in the treatment scheme for uncomplicated and mild cutaneous and complicated cutaneous and systemic cases of anthrax, ciprofloxacin and doxycycline are alternative agents [17]. In severe cases, the initial choice of antibiotic must be combined with one or two of the antibiotics such as penicillin, imipenem, ampicillin, meropenem, ciprofloxacin, rifampicin, clindamycin, aminoglycoside, linezolid or vancomycin [18].

Bacillus anthracis is resistant to late-stage cephalosporins such as cefoxime, cefotaxime, ceftazidime, aztreonam and trimethoprim-sulfomethoxazole [14]. In general, genes encoding acquired antibiotic resistance are found on mobile genetic elements such as transposons or plasmids. Through horizontal gene transfer, these elements can lead to the emergence of antibiotic resistance between Bacillus and other clinical pathogens [19]. It is a critical process to start treatment before *B. anthracis* begins to release toxins into the bloodstream. The use of a beta-lactam antibiotic such as penicillin is recommended by the Centers for Disease Control and Prevention (CDC) and the World Health Organisation (WHO) [20]. In developing countries where anthrax is endemic, penicillin drug is recognised as first choice for treatment due to its efficacy, widespread availability and low cost [21].

In this retrospective study, it was aimed to evaluate the antibiotic susceptibility, of *B. anthracis* isolates obtained from one human, various samples (blood, spleen, lung, liver, meat) of animals (cattle, sheep, dog, horse) that died from anthrax and soil samples taken from the animal burial areas between 2012 and 2023 from Kars province of Türkiye.

Material and Methods

Ethical Statement

This study vas approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: KAÜ-HADYEK/2023/15).

B. anthracis Isolates

A total of 87 *B. anthracis* isolates obtained from 1 human, various samples (blood, spleen, lung, liver, meat) of 61 animals (51 cattle, 7 sheep, 1 dog, and 2 horses) that died from anthrax and soil samples (25) taken from the animal burial areas brought to the Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University between 2012 and 2023 from Kars province, Türkiye, were used.

Isolation of *B. anthracis*

For isolation of *B. anthracis* from animal and human samples, 5% sheep blood agar was used. Medusa head-like and non haemolytic colonies grown after 24 h of incubation in aerobic coditions at 37ºC were evaluated. PLET Agar (Polymyxin B - Lysozyme - EDTA - Thallous acetate Agar) (Milipore, 55678) with Anthracis-Selective-Supplement (Milipore, 72659) was used for isolation of the agent from environmental samples. Petri dishes were incubated at 37°C for 36-40 h. The colonies that were circular, creamy-white with a ground glass texture were evaluated. Subcultured from this medium to blood agar was performed to confirm the isolates.

Phenotypical Identification

Bacillus anthracis suspected isolates were identified using the classical microbiological methods such as microscopic and macroscopic morphology, motility, and penicillin (10 U, Oxoid, UK) and gamma phage susceptibility [22].

Molecular Identification

DNA Extraction: A commercial extraction kit (56304, Qiagen, Germany) was used for the genomic DNA extraction of the *B. anthracis* isolates according to the manufacturer's instructions.

PCR Analysis: PCR targeting the amplification of capsule *(Cap)* and protective antigen *(PA)* genes was used for molecular identification of the *B. anthracis* isolates [22]. Amplification was performed using primers Cap6-5'-TACTGACGAGCAACCGA-3' and Cap103-5'-GGCTCAGTGTAACTCCTAAT-3', PA5-5'-GAGGTAG AAGGATACGGT-3' and PA8-5'-TCCTAACACTAACGA

AGTCG-3'. The PCR mixture was adjusted as 50 µL reaction volume including 25 µL Taq PCR Master Mix Kit (Qiagen, UK), 15 µL distilled water, 5 µL primer mix, and 5 µL template DNA. Thermal cycle conditions were as 30 cycles of initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 40 s, primer binding at 58°C for 40 s, elongation at 72°C for 40 s and final elongation at 72°C for 5 min. Amplified products were analysed by 1.5% gel electrophoresis (1640300, Bio-Rad, USA). Products of 1035 bp and 596 bp were considered positive for *Cap* and *PA* genes, respectively. *B. anthracis* Sterne strain lack of capsule was used as reference strain.

Antimicrobial Susceptibility Test

For determination the *in vitro* antimicrobial susceptibility of the *B. anthracis* isolates, the Kirby-Bauer disk diffusion method was applied [23]. Ten antimicrobials from 8 different groups including beta-lactams (penicillin [Oxoid, 10 U], amoxicillin [Oxoid, 25 µg]), sulfonamide (trimethoprim-sulfomethoxazole [Bioanalyse, 25 µg]), macrolide (erythromycin [Oxoid, 15 µg]), carbapenem (meropenem [Bioanalyse, 10 µg]), aminoglycoside (streptomycin [Oxoid, 10 µg]), fluoroquinolone (ofloxacin [Oxoid, 5 µg], ciprofloxacin [Oxoid, 5 µg]), fenicol (chloramphenicol [Oxoid, 30 µg]), lincosamide (clindamycin [Oxoid, 2 µg]) were used.

Bacterial inoculum was prepared from colonies of fresh *B. anthracis* cultures on 5% sheep blood agar in 0.9% physiological saline. Turbidity of the inoculum was adjusted to the 0.5 McFarland standard (approximately 10^8 cfu.mL^{-1}). 0.1 ml of bacterial inoculum was spread on Mueller Hinton agar and let to dry for 10 min. Then, antimicrobial discs were placed on the inoculated agar plates. The inhibition zone diameters formed after 24 h of incubation at 37ºC in air were evaluated. Inhibition zone was measured in milimeter using a ruler. Evaluations were made according to the standards of the Clinical and Laboratory Standards Institute (CLSI) [24], and the European Antibiotic Susceptibility Testing Committee (EUCAST) [25]. Since the inbition zone of *B. anthracis* in disk diffusion method has no been determined by CLSI, diameter of the Staphyloccal inhibition zone was used to interpretation. Since the breakpoints in EUCAST are given excluding *B. anthracis*, the evaluations were made using the breakpoints given for *Staphylococcus* spp. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains for the purpose of test reliability.

Statistical Analysis

The Pearson Chi Square test, one of the nonparametric tests, was used to evaluate the changes in the antibiotic susceptibility of *B. anthracis* strains according to sample origin and years.

Results

Phenotypic Identification Findings

All isolates were seen as encapsulated, large, square ended rods in tissue preparations, medusa head-like and non haemolytic colonies in 5% sheep blood agar, Typical circular, creamy-white with a ground glass texture colonies grown on Plet agar and Gram-positive bacterial cells with hair thread morphology in culture preparations. All of them were non-motile, susceptible to penicillin and gamma phage and showed mucoid and encapsulated colonies on bicarbonate agar. All of the isolates in this study were classified as *B. anthracis* according to the mentioned clasical phenotypic tests.

Molecular Identification Findings

As a result of *Cap* and *PA* specific PCR performed for the confirmation of the *B. anthracis* isolates, it was determined that all of them were virulent *B. anthracis* with the presence of fragments of 1035 and 596 bp for *Cap* and *PA* genes, respectively. *B. anthracis* Sterne showed fragment of 596 bp because of has not capsule gene *(Cap -, PA +) (Fig. 1)*.

Antimicrobial Susceptibility Findings

As a result of the disk diffusion method, all (100%) *B. anthracis* isolates were sensitive to penicillin, amoxicillin, ciprofloxacin and ofloxacin, whereas the sensitivity rate of the isolates to chloramphenicol, erythromycin, clindamycin, streptomycin, meropenem was found 95.4%,

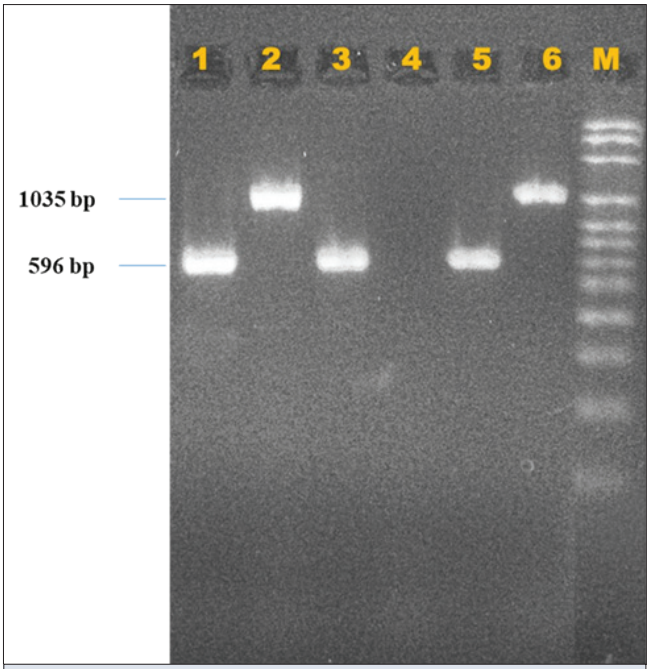

Fig 1. *PA* 5/8 (596 bp) and *Cap* 6/103 (1035 bp) PCR for *B. anthracis* confirmation. M: HyperLadder 100 bp Plus (Bioline); 1, 2, 5, and 6: *B. anthracis* field isolates; 3 and 4: *B. anthracis* Sterne strain

Table 1. *Antibiotic susceptibilities of 87 Bacillus anthracis strains isolated between 2012-2023*

Year	Origin	Antimicrobial Agents																														P Value
		P			AML			SXT			E			MEM			S			OFX			CIP			C			DA			
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
2012	Cattle	12	-	-	12	-	-	-	2	10	12	-	-	5	7	-	11	1	-	12	-	-	12	-	-	11	-	1	9	3	-	0.000
	Sheep	1	-	-	1	-	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
	Soil	8	-	-	8	-	-	-	-	8	8	-	-	3	5	-	8	-	-	8	-	-	8	-	-	7	1	-	5	3	-	0.000
2013	Cattle	12	-	-	12	-	-	-	2	10	10	1	1	4	8	-	8	4	-	12	-	-	12	-	-	12	-	-	11	1	-	0.000
	Sheep	1	-	-	1	-	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
	Dog	1	-	-	1	-	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
	Human	1	-	-	1	-	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
	Soil	9	-	-	9	-	-	-	-	9	9	-	-	4	5	-	8	1	-	9	-	-	9	-	-	8	1	-	7	2	-	0.000
2014	Cattle	9	-	-	9	-	-	-	1	8	8	1	-	5	4	-	9	-	-	9	-	-	9	-	-	8	1	-	8	1	-	0.000
	Soil	8	-	-	8	-	-	-	1	7	6	2	-	2	6	-	6	2	-	8	-	-	8	-	-	8	-	-	6	2	-	0.000
2015	Cattle	4	-	-	4	-	-	-	-	4	4	-	-	1	3	-	3	1	-	4	-	-	4	-	-	4	-	-	4	-	-	0.000
	Sheep	1	-	-	1	-	-	-	-	1	1	-	-	-	1	-	-	1	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
2016	Cattle	2	-	-	2	-	-	-	-	2	2	-	-	-	2	-	1	1	-	2	-	-	2	-	-	2	-	-	2	-	-	0.000
2018	Cattle	3	-	-	3	-	-	-	-	3	3	-	-	-	3	-	3	-	-	3	-	-	3	-	-	3	-	-	3	-	-	0.000
	Sheep	2	-	-	2	-	-	-	-	2	2	-	-	-	2	-	1	1	-	2	-	-	2	-	-	2	-	-	2	-	-	0.000
	Horse	2	-	-	2	-	-	-	-	2	1	-	1	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	2	-	-	0.000
2019	Cattle	4	-	-	4	-	-	-	-	4	3	1	-	-	4	-	3	1	-	4	-	-	4	-	-	4	-	-	4	-	-	0.000
	Sheep	1	-	-	1	-	-	-	-	1	1	-	-	-	1	-	-	1	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
2020	Cattle	2	-	-	2	-	-	-	-	2	2	-	-	-	2	-	1	1	-	2	-	-	2	-	-	2	-	-	2	-	-	0.000
2021	Cattle	1	-	-	1	-	-	-	-	1	1	-	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
2022	Cattle	2	-	-	2	-	-	-	-	2	1	-	1	2	-	-	1	1	-	2	-	-	2	-	-	2	-	-	2	-	-	0.000
2023	Sheep	1	-	-	1	-	-	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-	0.000
Total (%)		**87 (100)**	-	-	**87 (100)**	-	-	-	**6 (6.9)**	**81 (93.1)**	**79 (91)**	**5 (6)**	**3 (3)**	**33 (38)**	**53 (61)**	**1 (1)**	**71 (82)**	**16 (18)**	-	**87 (100)**	-	-	**87 (100)**	-	-	**83 (95.4)**	**3 (3.4)**	**1 (1.1)**	**75 (86)**	**12 (13.8)**	-	

P: Penicillin; AML: Amoxicillin, SXT: Trimethoprim-sulfometoxazole, E: Erythromycin; MEM: Meropenem; S: Streptomycin; OFX: Ofloxacin; CIP: Ciprofloxacin; C: Chloramphenicol; DA: Clindamycin; S: Susceptible; I: Intermediate; R: Resistant

Table 2. *Antibiogram test results as percent and origins of B. anthracis strains*

Antimicrobial Agents	Zone Diameter Breakpoints (mm)			Origin and Percent of Isolates																	
	S (≥)	I	R (≤)	Cattle (n: 51)			Sheep (n: 7)			Dog (n: 1)			Horse (n: 2)			Human (n: 1)			Soil (n: 25)		
				S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
P[a]	29	-	28	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
AML[b]	26	-	26	100	-		100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
SXT[a]	16	11-15	10	-	9.8	90.2	-	-	100	-	-	100	-	-	100	-	-	100	-	4	96
E[a]	23	14-22	13	90	6	4	100	-	-	100	-	-	50	-	50	100	-	-	92	8	-
MEM[a]	22	-	22	33	65	2	43	57	-	100	-	-	100	-	-	100	-	-	36	64	-
S[a]	22	-	14	80.4	19.6	-	57	43	-	100	-	-	100	-	-	100	-	-	88	12	-
OFX[a]	18	15-17	14	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
CIP[a]	21	16-20	15	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
C[a]	18	13-17	12	96	2	2	100	-	-	100	-	-	100	-	-	100	-	-	92	8	-
DA[a]	21	15-20	14	90.2	9.8	-	100	-	-	100	-	-	100	-	-	100	-	-	72	28	-

[a]*According to S. aureus inhibition zone from CLSI M100-30th ed.*[24],
[b]*According to S. aureus inhibition zone from EUCAST- Version 13.1* [25],
P: Penicillin; AML: Amoxicillin, SXT: Trimethoprim-sulfometoxazole, E: Erythromycin; MEM: Meropenem; S: Streptomycin; OFX: Ofloxacin; CIP: Ciprofloxacin; C: Chloramphenicol; DA: Clindamycin; S:Susceptible; I: Intermediate; R: Resistant

91%, 86%, 82%, and 38%, respectively. Out of the isolates 61% were moderately susceptible to meropenem, 18% to streptomycin, 13.8% to clindamycin, 6.9% to trimethoprim-sulfomethoxazole, 6% to erythromycin and and 3.4% to chloramphenicol. Among the isolates, 93.1% of them were found to be resistant to trimethoprim-sulfometoxazole, 3% to erythromycin, 1.1% to chloramphenicol and 1% to meropenem. *Table 1* displays antibiotic susceptibilities of 87 *B. anthracis* strains isolated between 2012-2023.

When the antimicroabial susceptibilities of the *B. anthracis* isolates were evaluated according to their origin, it was observed that while dog and human origin isolates were susceptible to all antibiotics (except trimethoprim-sulfomethoxazole), all of bovine and soil origin isolates were susceptible to penicillin, amoxicillin, ofloxacin and ciprofloxacin antibiotics, all sheep origin isolates were susceptible to penicillin, amoxicillin, ofloxacin, erythromycin, ciprofloxacin, clindamycin, and chloramphenicol antibiotics, all horse origin isolates were susceptible to penicillin, amoxicillin, meropenem, streptomycin, ofloxacin, ciprofloxacin, chloramphenicol and clindamycin. Erythromycin susceptibility rates were 90.2%, 90% and 50% in soil, cattle and horse isolates, respectively; streptomycin susceptibility rates were 88%, 80.4% and 57% in soil, cattle and sheep isolates, respectively; chloramphenicol susceptibility rates were 96% and 92% in cattle and soil isolates; chloramphenicol susceptibility rates were 96% and 72% in cattle and horse isolates, respectively. In addition, 65% of the bovine origin isolates were moderately susceptible to meropenem and 9.8% to trimethoprim-sulfomethoxazole, and 64% of the soil origin isolates were moderately susceptible to meropenem and 4% to trimethoprim-sulfomethoxazole. All sheep, horse, dog and human isolates, 90.2% of bovine isolates and 96% of soil isolates were resistant to trimethoprim-sulfomethoxazole. Antibiogram test results as percent and origins of *B. anthracis* strains were given in *Table 2*.

When antimicrobial susceptibilities are analysed according to years, it was seen that cattle, sheep, and soil isolates were susceptible to penicillin, amoxicillin, ofloxacin and ciprofloxacin in all years tested (2012-2023). All sheep origin isolates were also susceptible to chloramphenicol, erythromycin, and clindamycin and resistant to trimethoprim-sulfomethoxazole in all years. Bovine isolates were also susceptible to cholaramphenicol and erythromycin in 2015 and later years. While in 2012, 2013 and 2014 years, 83.3%, 83.3% and 88.9% of bovine isolates were resistant to trimethoprim-sulfomethoxazole, 16.7%, 16.7% and 11.1% were moderately susceptible, respectively. All isolates identified in 2015 and subsequent years were resistant to trimethoprim-sulfomethoxazole. Dog (1 isolate from 2013), human (1 isolate from 2013)

and horse (2 isolates from 2018) isolates were susceptible to all antimicrobial tested. Just 1 horse isolate was resistant to erytromycin. Antibiotic susceptibilities of dog, human, and horse isolates were presented in *Table 1*.

Statistical Analysis Findings

At a result of the Chi-Square analysis ($\chi 2$ = 141.897; P=0.000), a statistically significant difference in the antibiotic susceptibility of *B. anthracis* strains according to sample origin and years was found.

Discussion

Bacillus anthracis is sensitive to many antibiotics such as penicillin, oxytetracycline, amoxicillin, chloramphenicol, ciprofloxacin, doxycycline, erythromycin, gentamicin and sulphonamides [3]. Among these, penicillin is used both in the diagnosis of the agent and in the treatment of the disease [26]. Penicillin is the first preferred antibiotic in anthrax treatment [11,27]. All 87 *B. anthracis* strains used in the present study were found to be susceptible to penicillin. This result is consistent with the studies of Aydın et al.[26], Doğanay and Aydın [27], Eşel et al.[28], Otlu et al.[29], Chun et al.[30], Habrun et al.[31], and Perçin et al.[32].

The drugs preferred for post-exposure prophylaxis of anthrax include amoxicillin, ciprofloxacin or ofloxacin as well as penicillin G [33]. Also oral amoxicillin may be used in mild, uncomplicated cutaneous anthrax [2]. In addition ciprofloxacin is one of the first choices in bio-terrorism or biological weapon related anthrax. Amoxicillin, ofloxacin and ciprofloxacin evaluated in the present study showed very good activity on all *B. anthracis* strains. This result is consistent with the studies of Doğanay and Aydın [27], Eşel et al.[28], Chun et al.[30], Habrun et al.[31] and Cavallo et al.[34]. As can be seen, *B. anthracis* remains susceptible to amoxicillin, ofloxacin and ciprofloxacin. According to these results obtained, it is thought that it may be significant to continue to include these antibiotics in the treatment prescription in clinical cases.

In patients with penicillin allergy, erythromycin, streptomycin, chloramphenicol and clindamycin are among the alternative drugs that can be used in the treatment of anthrax [5,35]. Also, in systemic anthrax, penicillin must be combined with one or two antibiotics to which the bacteria are susceptible. In cases of pulmonary anthrax, penicillin G must be combined with clindamycin or ciprofloxacin, and in gastrointestinal anthrax, it must be combined with aminoglycoside (preferably streptomycin) [2]. The penicillin drug combined with streptomycin is also curative [3]. In the present study, 95.4% of the strains were susceptible to chloramphenicol, 91% to erythromycin, 86% to clindamycin and 82% to streptomycin. Effectivity of these antibiotics on all *B. anthracis* strains tested suggest that there may be several suitable alternative antimicrobial agents for the prophylaxis and/or treatment of *B. anthracis*.

In anthrax meningitis, which is a life-threatening clinical picture, a combination of quinolone (such as ciprofloxacin) + carbapenem (such as meropenem) + a protein synthesis inhibitor antibiotic (such as clindamycin/chloramphenicol) can be used [36]. Another antibiotic used in the present study was meropenem. As a result of the study, 33% of the strains were susceptible to meropenem, 66% were moderately susceptible and 1% were resistant. According to this result of the present study, it is useful to pay attention to results of antibiogram to be performed in case of the use of meropenem and to organise the treatment accordingly.

B. anthracis has been found to develop high rates of resistance to trimethoprim-sulfomethoxazole [2,5]. Although not associated with sulfoamethoxazole, trimethoprim resistance has been reported in some studies [7,31,37,38]. In the present study, resistance to trimethoprim-sulfomethoxazole was determined in 93.1% of the isolates and this result is in parallel with the other studies [7,39]. This result supports studies reporting that *B. anthracis* has an intrinsic resistance to this antibiotic [40], and the claim that this antibiotic should not be used in anthrax prophylaxis or treatment in humans [28]. Therefore, bacterial antimicrobial resistance, which reduces the efficacy of drugs used to treat bacterial infections, is therefore a major public health problem and studies on the mechanisms and spread of antibiotic resistance are very important [41].

Before deciding on treatment, it should be remembered that *B. anthracis* has developed resistance to trimethoprim-sulfomethoxazole as well as third generation cephalosporins. Aydın et al [26], in a study on the number of anthrax cases in humans and animals in Kars region between 1995 and 2000, determined that all of the 61 *B. anthracis* strains (45 cattle, 6 sheep and 10 human origin) were susceptible to trimethoprim-sulfomethoxazole. Unlike from this, Otlu et al.[29], reported that all 61 cattle and 13 sheep *B. anthracis* isolates were resistant to trimethoprim in their study on the antibimicrobial susceptibility/resistance of sheep and cattle origin *B. anthracis* strains raised in Kars and Ardahan regions. In the present study, while 16.7%, 16.7% and 11.1% of the bovine *B. anthracis* isolates identified in 2012, 2013, and 2014 were moderately susceptible to trimethoprim-sulfomethoxazole, respectively, all bovine isolates identified in 2015 and later years were resistant to trimethoprim-sulfomethoxazole. This result can be considered as an indication that *B. anthracis* strains develop intrinsic resistance to SXT as mentioned above.

Anthrax is one of the serious diseases in animals and humans throughout history and remains a major zoonotic

concern. Timely recognition of *B. anthracis* infection is essential for determination of appropriate treatment, identification of outbreaks and veterinary and public health interventions. Therefore, in determining preventive and therapeutic strategies including the using of antibiotics in anthrax, which carries a high risk for public health, it will be useful further monitoring *B. anthracis* with the consideration of its resistance and susceptibility profile and to decide on the best therapeutic strategy (current or alternative treatment options) considering the results of antibiotic susceptibility test. The results showed no change in the susceptibility profile of *B. anthracis* isolates, especially penicillin and amoxicillin susceptibility profiles which are the first antimicrobial agents preferred in naturally occurring anthrax cases of *B. anthracis* strains in Kars region in the current study.

Anthrax is important as a potential weapon of bioterrorism. Therefore, if the antimicrobial resistance of the causative agent *B. anthracis* increases, the treatment of this pathogen in bioterrorism events may become more difficult. In particular, the development of resistance to potent and broad-spectrum antibiotics could pose a major threat to public health. In cases of increasing antibiotic resistance, the need for more complex treatment strategies, the use of antitoxins, combination therapies and rapid diagnostic methods may also increase. Ciprofloxacin, one of the first choice antibiotics in anthrax associated with biological weapons or bioterrorism, was found to be fully effective against *B. anthracis* in the present study. However, as mentioned above, the possibility that the resistance to trimethoprim-sulfomethoxazole antibiotic may similarly develop against ciprofloxacin and penicillin antibiotic, which is the first choice in prophylaxis and treatment protocols, should not be ignored. Therefore, in order to be prepared for resistance in possible bioterrorism attacks, there is an intense need for practices for monitoring antibiotic susceptibility/resistance, comprehensive treatment strategies and strong integration of effective interventions for public health. Conclusion, changes in the antimicrobial susceptibility of *B. anthracis* significantly affect treatment options in bioterrorism events, which can make rapidly spreading infections more difficult to control. Therefore, it is important to develop more comprehensive treatment strategies, faster and more accurate diagnostic methods, and effective intervention protocols against bioterrorism events. But as of right now, athough *B. anthracis* strains are sensitive to certain antibiotics, it is important that drugs should not be used in anthrax prophylaxis or medical and veterinary management without prior susceptibility testing.

Considering the results obtained from the current study, it is thought that further studies involving standardized testing methodologies for the antibiotic resistance profile of *B. anthracis* and the continuation of these studies will elucidate the resistance mechanisms that may develop in such a bioterrorism agent.

Declerations

Availability of Data and Materials: The data and materials of this study are available from the corresponding author (E. Çelik).

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Author Contributions: EÇ: Design of study; isolation and identification of isolates, PCR analysis, writing - review & editing; AGS: Design of study, isolation and identification of isolates, PCR analysis; FB: Design of study, evaluation the results, review; SO: Design of study, evaluation the results, review; MŞ: Design of study, evaluation the results, review; ÖÇ: Design of study, evaluation the results; MRC: Isolation and identification of isolates, antibiotic susceptibility testing; SG: Isolation and identification of isolates, antibiotic susceptibility testing; YE: Isolation and identification of isolates, antibiotic susceptibility testing; EB: Isolation and identification of isolates, antibiotic susceptibility testing; BE: Antibiotic susceptibility testing.

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