

The Sensitivity Tests of Beta-Lactamase Positive and Negative of *Bacillus* Isolates Against Clarithromycin, Azithromycin, I. and III. Class Cephalosporin and Amicasin Antibiotics

Güven URAZ*^o, Mine KAANOĞLU*, Ünal KOL*

The Sensitivity Tests of Beta-Lactamase Positive and Negative of Bacillus Isolates Against Clarithromycin, Azithromycin, I. and III. Class Cephalosporin and Amicasin Antibiotics

Summary : A total of, 33 *Bacillus* strains were isolated from different raw milk samples. In this research, the activity of beta-lactamase enzyme of *Bacillus* strains was evaluated by Iodometric Test and Chromogenic Cephalosporin Test (Nitrocefın). The *Bacillus* species which were determined as beta-lactamase positive by using Iodometric Test and Chromogenic Cephalosporin Test (Nitrocefın) were then controlled by penicillin-G antibiotic. The 10 samples of 33 *Bacillus* strains determined to be positive for activity of beta-lactamase enzyme. The two strains of 10 *Bacillus* strains which showed beta-lactamase positive activity were *B.lentus*, one strain was *B.megaterium*, two strains were *B.brevis*, two strains were *B.mycoides* and three strains were *B.polymxa*. The beta-lactamase activity of the remaining 23 *Bacillus* isolates was found to be negative. One of these was *B.brevis*, three strains were *B.sphaericus*, three strains were *B.cereus*, two strains were *B.circulans*, two strains were *B.coagulans*, three strains were *B.licheniformis*, two strains were *B.macerans*, one strain was *B.mycoides*, three strains were *B.stearothermophilus* and three strains were *B.subtilis*. Also, the sensitivity tests of beta-lactamase positive and negative *Bacillus* strains were made against the antibiotics such as azithromycin, clarithromycin, Ist, and IIIrd. class cephalosporin and amicasin antibiotics. Beta-lactamase positive and negative *Bacillus* strains were found to be sensitive to azithromycin and clarithromycin. *B.megaterium*, *B.polymxa-II*, *B.polymxa-III*, *B.mycoides-I* and *B.brevis-I* strains from 10 beta-lactamase positive *Bacillus* species were found to be resistant against ceftriaxone antibiotic which is a III.class cephalosporine. The others, such as *B.lentus-I*, *B.polymxa-I*, *B.mycoides-II* and *B.brevis-II* were found to be sensitive. *B.lentus*, *B.polymxa-I*, *B.mycoides-II* and *B.brevis-II* which were positive of beta-lactamase from *Bacillus* species were found to be sensitive to cephalosporin antibiotic which is a Ist. class cephalosporine. The others were determined to be resistant *B.lentus-I*, *B.lentus-II* and *B.mycoides-II* were found to be sensitive to cephalothine antibiotic. The remaining ones were found to be resistant. Only one strain, *B.mycoides-I* from the beta-lactamase positive *Bacillus* strains was determined to be resistant against amicasin antibiotic. The remaining ones were found to be sensitive. All beta-lactamase

Klaritromisin, Azitromisin, I. ve III. Kuşak Sefalosporin Grubu ve Amikasin Antibiyotiklerine Karşı Beta-Laktamaz Pozitif ve Negatif Bacillus'ların Duyarlılıkları

Özet : Çiğ süt örneklerinden toplam 33 *Bacillus* izole edilmiştir. Araştırmada izole edilen *Bacillus* türlerinin beta-laktamaz enzim aktiviteleri Iodometrik Test ve Kromojenik Sefalosporin (Nitrocefın) Test yöntemleri ile çalışılarak değerlendirilmiştir. Beta-laktamaz pozitif olarak tespit edilen *Bacillus* türleri daha sonra penisilin-G antibiyotiği ile de kontrol edilmiştir. Toplam 33 *Bacillus*'un 10'unda beta-laktamaz enzim aktivitesi pozitif olarak tespit edilmiştir. Bu *Bacillus*'ların 2'si *B.lentus*, 1'i *B.megaterium*, 2'si *B.brevis*, 2'si *B.mycoides* ve 3'ü de *B.polymxa*'dır. Geriye kalan 23 *Bacillus* izolatının beta-laktamaz enzim varlığı negatif olarak tespit edilmiştir. Beta-laktamaz enzim varlığı negatif *Bacillus*'ların 1'i *B.brevis*, 3'ü *B.sphaericus*, 3'ü *B.cereus*, 2'si *B.circulans*, 2'si *B.coagulans*, 3'ü *B.licheniformis*, 2'si *B.macerans*, 1'i *B.mycoides*, 3'ü *B.stearothermophilus* ve 3'ü de *B.subtilis*'dir. Ayrıca, beta-laktamaz pozitif ve negatif *Bacillus* izolatlarının klaritromisin, azitromisin, I. ve III. kuşak sefalosporin ve amikasin antibiyotiklerine karşı hassasiyet testleri de yapılmıştır. Çalışma sonucunda beta-laktamaz pozitif ve negatif *Bacillus* türleri, azitromisin ve klaritromisine duyarlı tespit edilmiştir. 10 beta-laktamaz pozitif *Bacillus* türünden *B.megaterium*, *B.polymxa-II*, *B.polymxa-III*, *B.mycoides-I* ve *B.brevis-I*'in III. kuşak sefalosporin grubundan olan seftriaksona karşı dirençli geriye kalanların ise duyarlı oldukları saptanmıştır. I. kuşak sefalosporin grubundan olan sefazoline karşı beta-laktamaz pozitif *Bacillus* türlerinden *B.lentus*, *B.polymxa-I*, *B.mycoides-II* ve *B.bservis-II*'nin duyarlı diğerlerinin dirençli oldukları görülmüştür. Sefalotine karşı sadece beta-laktamaz pozitif *B.lentus-I*, *B.lentus-II* ve *B.mycoides-II* türleri duyarlı olarak bulunmuş, geriye kalan diğer beta-laktamaz pozitif türler dirençli olarak tespit edilmiştir. Beta-laktamaz pozitif *Bacillus* türlerinden amikasine karşı sadece *B.mycoides-I* dirençli diğerleri duyarlı olarak tespit edilmiştir.

Beta-laktamaz negatif Bacillus türlerinden, seftriaksona

* Gazi University, Faculty of Science and Arts, Department of Biology, 06500 Teknikokullar-Beşevler, Ankara, Turkey

^o Correspondence

negative *Bacillus* strains except for *B.licheniformis* were found to be sensitive to ceftriaxone antibiotic. *B.brevis-III*, *B.licheniformis*, *B.stearothermophilus* and *B.sphaericus* from the beta-lactamase negative *Bacillus* strains were found to be resistant to cephalothine antibiotic, the others were found to be sensitive. Only one strain, *B.licheniformis* from the beta-lactamase negative *Bacillus* strains was found to be resistant to cephazoline antibiotic. The others were found to be sensitive. Only one strain, *B.cereus* from the beta-lactamase negative *Bacillus* strain, was found to be resistant to amicasine antibiotic.

Key words: *Bacillus*, Beta-lactamase enzyme.

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INTRODUCTION

In recent years, studies related with the beta-lactamase enzyme of *Bacillus* strains have become numerous in literature¹. The researchers have shown the beta-lactamase enzyme bounded with plasmides which was synthesized by *Bacillus* species. These are *B.cereus*, *B.subtilis*, *B.licheniformis*, *B.stearothermophilus*². In 1983, Nielsen and his colleagues have determined that the enzyme of *B.cereus* 569 strain was similar to β -penicillinase and they were named beta-lactamase III. It has been observed that the *B.cereus* 569 strains, except for *B.cereus* 5/B, produced beta-lactamase III enzyme³.

In 1991, Turnbull and Kramer have found that *B.cereus* and *B.thuringiensis* produced beta-lactamase enzyme. The species of *Bacillus* found to be resistant to penicilline, ampicilline and cephalosporines. Meanwhile, the species of *Bacillus* were found to be sensitive to tetracycline, sulphonamide, clindamycine, erytromycine, chloramphenicol, vancomycine and aminoglycoside antibiotics⁴.

In the research of the activity of beta-lactamase enzyme, various methods are used. The most widely used methods are Iodometric Test and Chromogenic Cephalosporine Test to determine the enzyme activity of beta-lactamase.

In 1991, Nakamura and his colleagues used the Iodometric Test to determine the enzyme activity of beta-lactamase in research on the mutants of *B.subtilis*⁵.

In 1991, Hemilia and Sibakov preferred the spectrophotometric method by using nitrocefine as

karşı *B.licheniformis* türü hariç tüm *Bacillus* türleri hassasiyet göstermiştir. Sefalotine karşı beta-laktamaz negatif *B.brevis-III*, *B.licheniformis*, *B.stearothermophilus* ve *B.sphaericus* türleri dirençli, diğerleri duyarlı olarak tespit edilmiştir. Sefazoline karşı sadece beta-laktamaz negatif *B.licheniformis* türü dirençli, diğerleri duyarlı olarak tespit edilmiştir. Amikasine karşı ise sadece beta-laktamaz negatif *Bacillus B.cereus* türü dirençli olarak tespit edilmiştir.

Anahtar kelimeler: *Bacillus*, Beta-laktamaz enzimi.

substrate to determine beta-lactamase activity in the studies of heterolog proteins of *B.subtilis*⁶.

In 1996, Uraz and her colleagues used the Iodometric Test to determine beta-lactamase activity of *Bacillus* strains. They have found that 30 of 72 *Bacillus* strains showed positive beta-lactamase activity⁷.

We isolated and named *Bacillus* strains by using findings of the researches mentioned above and studied the activity of beta-lactamase enzyme. The positive and negative activity of beta-lactamase of *Bacillus* isolates were studied as sensitive to clarithromycin, azithromycin, I. and III. class cephalosporine and amicasin antibiotics. Although our samples were not of human origin, the nourishment pathogens were taken within the *Bacillus* strains studied. Especially in recent literature, *B.cereus* and the other *Bacillus* species have gained great importance as the nourishment pathogens. For this reason, we aimed to determine the beta-lactamase and antibiotic relationship of *Bacillus* species.

MATERIAL AND METHOD

In this research, a total of 33 *Bacillus* strains from raw milk samples were isolated and named. PCA (Plate Count Agar) and Glucose Agar were used for isolating *Bacillus* strains (Laberau and Mair, 1968)⁸. *Bacillus* colonies grown on these plates were purified on Glucose Agar after the biochemical tests were done.

In the naming of *Bacillus* strains, the various biochemical tests such as catalase production, gelatine hydrolyse, the use of citrate, TSI, urease, indole, VP,

movement (SIM), reduction of nitrate and hemolysis in blood agar were used. Since the aim of the test was to use the capability of various sugars of *Bacillus* strains, the fermentation tests were studied. The sugars used are glucose, xylose, mannitol, lactose, sucrose and maltose. The carbohydrate fermentation medium (Purple Broth Base-Difco) was used for this purpose^{4,9,13,14}. The activities of beta-lactamase of *Bacillus* strains were evaluated by using two different tests, Iodometric Test and Chromogenic Cephalosporine Test (Nitrocefine) (Beta-Lactamase Identification Sticks Oxoid BR 66, Dry-Slight Nitrocefine Difco Laboratories 3537). In the results of both tests, the antibiogramme tests with penicillin-G were carried out on the bacteria for positivity and controlled as being resistant to penicillin-G. Later, the positive and negative activities of beta-lactamase enzyme of *Bacillus* strains were studied for sensitivity to clarithromycin, azithromycin, I. and III. class cephalosporine and ampicillin. The antibiogrammes were evaluated using the Kirby-Bauer Disc Diffusion method¹⁰⁻¹⁴.

In the sensitivity tests for antibiotics, the Mueller-Hinton Medium was used. The sensitive and resistant values for clarithromycin were 6-13mm \geq / \geq 18mm, the values for azithromycin were 6-13mm \geq / \geq 18mm, the values for ceftriaxone were 13mm \geq / \geq 21 mm, the values for ampicillin were 14mm \geq / \geq 17mm, the values for cephalothin were 14mm \geq / \geq 18 mm, the values for cephazolin were 14mm \geq / \geq 18 mm and

the values for penicillin-G were 19mm \geq / \geq 28mm. The results were calculated according to these values¹⁰.

Several colonies were taken from each tested strain and these were incubated in the medium tubes containing 4 ml Nutrient Broth. They were incubated at 37°C for 24 hours. The turbidity was compared with the Turbidity Standard for determining the bacteria density (Turbidity Standard : 0,5 ml. 0,048 M BaCl₂ (11,7 gr/1 BaCl₂ .2H₂O) :99,5 ml, %1 H₂SO₄ v/v (0,36 N)). The inoculation was made from liquid cultures of each tubes (the bacteria number adjusted) to Mueller-Hinton medium in petri dishes. Afterwards, the antibiotics in sterile conditions were placed on plates in turn. After incubation at 37°C for 24 hours, the zone diameters surrounding the discs were measured an by a mm ruler¹⁰.

RESULTS

Total, 33 *Bacillus* strains were isolated from raw milk samples. In the research, *Bacillus* strains widely seen were 3 *B.brevis* (9%), 3 *B.cereus* (9%), 3 *B.licheniformis* (9%), 3 *B.mycooides* (9%), 3 *B.sphaericus* (9%), 3 *B.stearothermophilus* (9%) and 3 *B.subtilis* (9%). The isolation of *B.megaterium* (3%) was the least seen. The beta-lactamase enzyme activities of *Bacillus* strains were given as the comparative results of Iodometric Test and Chromogenic Cephalosporin Test (Nitrocefine) in Table 1.

Table 1. The number and distribution of positive and negative beta-lactamase enzyme of *Bacillus* strains.

<i>Bacillus species</i>	Using Iodometric and Nitrocefine Test (Beta-Lactamase Identification Stick Oxoid BR 66 number", "DrySlight NITROSEFIN DIFCO LABORATORIE 3537"), The Determination of beta-lactamase enzyme	
	positive <i>Bacillus</i> number	negative <i>Bacillus</i> number
<i>Bacillus lentus</i>	2	
<i>Bacillus circulans</i>		2
<i>Bacillus subtilis</i>		3
<i>Bacillus macerans</i>		2
<i>Bacillus cereus</i>		3
<i>Bacillus coagulans</i>		2
<i>Bacillus licheniformis</i>		3
<i>Bacillus sphaericus</i>		3
<i>Bacillus megaterium</i>	1	
<i>Bacillus brevis</i>	2	1
<i>Bacillus mycooides</i>	2	1
<i>Bacillus polymxa</i>	3	
<i>Bacillus stearothermophilus</i>		3
TOTAL	10	23

We have found the same results of the former method. *B.brevis* in two, *B.lentus* in two, *B.mycoides* in two, *B.polymxa* in three and *B.megaterium* in one of 10 *Bacillus* strains determined as positive beta-lactamase were found in both methods. *B.cereus* in three, *B.licheniformis* in three, *B.sphaericus* in three, *B.stearothermophilus* in three, *B.subtilis* in three, *B.circularans* in two, *B.coagulans* in two, *B.macerans* in two and, *B.brevis* in one, and *B.mycoides* in one of 23 *Bacillus* strains determined as negative beta-lactamase were found. The results of both methods have supported each other. By addition of the two methods, positive and negative beta-lactamase activities of *Bacillus* strains were confirmed by using the test of penicillin-G antibiotic. As a result, the *Bacillus* strains determined positive beta-lactamase were found to be 100% resistant to penicillin-G antibiotic.

Afterwards, the sensitivities of *Bacillus* strains found to have positive beta-lactamase enzyme activities were studied against clarithromycin, azithromycin, I. and III. Class cephalosporine and amicasin antibiotics by using the method of Kirby-Bauer disc diffusion. By this aim, the zone diameters of sensitive *Bacillus* strains against the antibiotics were evaluated (Figures 1,2,3,4).

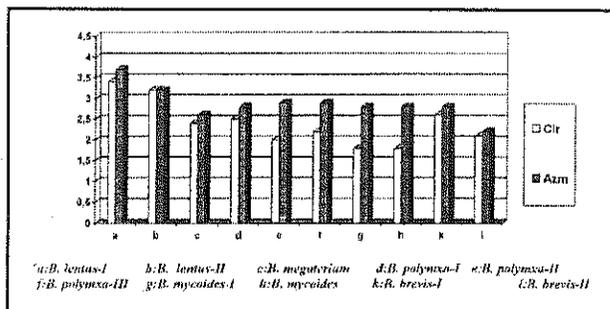


Figure 1. The sensitivities of *Bacillus* species evaluated positive beta-lactamase against Clarithromycin (Clr) and Azithromycin (Azm)

In all *Bacillus* species such as *B.lentus*-I, *B.lentus*-II, *B.megaterium*, *B.polymxa*-I, *B.polymxa*-II, *B.polymxa*-III, *B.mycoides*-I, *B.mycoides*-II, *B.brevis*-I and *B.brevis*-II evaluated positive beta-lactamase, the zone diameters of azithromycin were much larger than that of clarithromycin, but both antibiotics were found to be effected.

B.brevis-III, *B.cereus*, *B.circularans*, *B.coagulans*, *B.licheniformis*, *B.macerans*, *B.mycoides*-II,

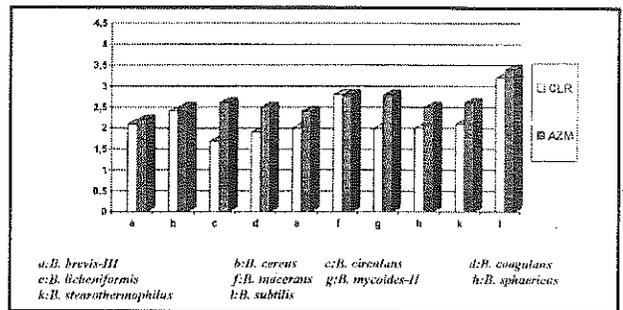


Figure 2. The sensitivities of *Bacillus* species evaluated negative beta-lactamase against Clarithromycin (Clr) and Azithromycin (AZM)

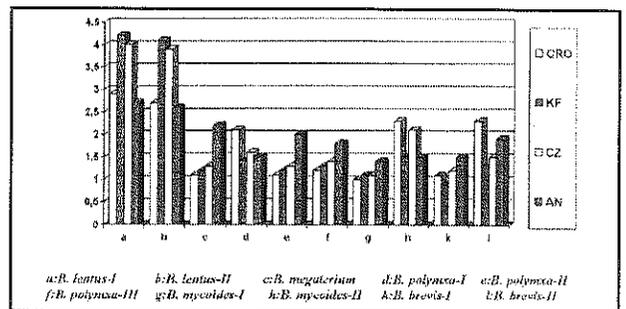


Figure 3. The sensitivities and resistances of *Bacillus* species evaluated positive beta-lactamase activity against Ceftriaxone (Cro), Cephalothin (KF), Cephalozin (CZ) and Amicasin (AN) antibiotics

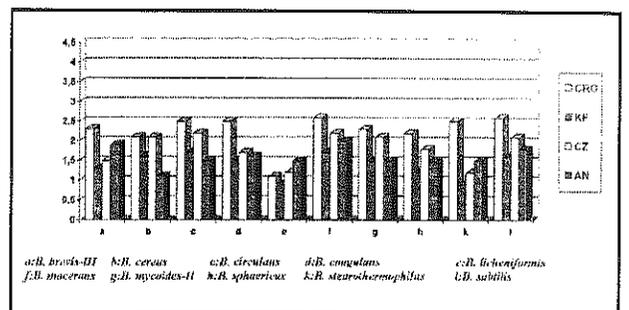


Figure 4. The sensitivities and resistances of *Bacillus* species evaluated negative beta-lactamase activity against Ceftriaxone (Cro), Cephalothin (KF), Cephalozin (CZ) and Amicasin (AN) antibiotics

B.sphaericus, *B.stearothermophilus* and *B. subtilis* of *Bacillus* strains found to have negative beta-lactamase activity were sensitive against clarithromycin and azithromycin antibiotics. As a result, clarithromycin and azithromycin were determined effective against all *Bacillus* isolates.

In our research, the group of cephalosporine antibiotics (Ceftriaxone, Cephalozin and Cephalothin)

were effective against *Bacillus* strains. *B.megaterium*, *B.polymxa-II*, *B.polymxa-III*, *B.mycooides-I* and *B.brevis-I*, evaluated as positive beta-lactamase activity, were determined to be resistant to ceftriaxone from IIInd.class cephalosporine and the other species were found to be sensitive. *B.lentus*, *B.polymxa-I*, *B.mycooides-II* and *B.brevis-II*, evaluated as positive beta-lactamase activity, were found to be sensitive to cefazoline from Ist.class cephalosporine and the other species were found to be resistant. Cephalothin antibiotic was found to be effective against *B.lentus-I*, *B.lentus-II* and *B.mycooides-II*. Only *B.mycooides-I* were resistant against amicasin. *B.brevis-I*, *B.brevis-II*, *B.lentus-I*, *B.lentus-II*, *B.megaterium*, *B.polymxa-I*, *B.polymxa-II*, *B.polymxa-III* were sensitive to the same antibiotic.

All *Bacillus* species except for *B.licheniformis* with negative beta-lactamase activity were shown to be sensitive to ceftriaxone antibiotic. *B.brevis-III*, *B.licheniformis*, *B.stearothermophilus* and *B.sphaericus* were resistant to cephalothin antibiotic. Only *B.licheniformis* was resistant to cephalozin antibiotic. The others were sensitive to cephalozin antibiotic. Only *B.cereus* was resistant to amicasin antibiotic.

DISCUSSION

Bacillus strains which have been gained a new importance such as nourishment pathogens have produced an important enzyme for clinicals as beta-lactamase. Beta-lactamase has inhibited beta-lactam antibiotics.

In the research, the activities of beta-lactamase enzyme of *Bacillus* species were studied by using Iodometric Test and Chromogenic Cephalosporine Test (Nitrocefine). The both tests have been found to be reliability at the same ratio for determining the enzyme.

In 1984, Tal and his colleagues were studied by using Iodometric Test for the activity of beta-lactamase enzyme of *Bacillus* species¹⁵. We used the same method. Besides we used Chromogenic Cephalosporine Test (Nitrocefine).

In 1992, Baker was researched the activity of beta-

lactamase enzyme of *Bacillus* species by using Chromogenic Cephalosporine Test (Nitrocefine)¹⁶.

In our research, *Bacillus* species determined positive and negative beta-lactamase enzyme activities were tested to sensitive against clarithromycin, azithromycin, I. and III. class cephalosporine and amicasin antibiotics. The macrolid group of 10 *Bacillus* species determined positive activity were showed to be sensitive against clarithromycin and azithromycin which are the new medicines. Besides, *Bacillus* species *B.megaterium*, *B.polymxa-II*, *B.polymxa-III*, *B.mycooides-I* and *B.brevis-I* were determined to be resistant against ceftriaxone antibiotic from the III.class cephalosporine. The others, *B.lentus*, *B.polymxa-I*, *B.mycooides-II* and *B.brevis-II* were determined to be sensitive against ceftriaxone. *B.megaterium*, *B.polymxa-II*, *B.polymxa-III*, *B.mycooides-I* and *B.brevis-I* were determined to be resistant against cephalozin antibiotic from the I.class cephalosporine. *B.lentus*, *B.polymxa-I*, *B.mycooides-II* and *B.brevis-II* were determined to be sensitive against the same antibiotic. Furthermore, *B.brevis*, *B.mycooides*, *B.megaterium*, *B.polymxa* were determined to be resistant against cephalothin antibiotic. The others, *B.lentus-I*, *B.lentus-II* and *B.mycooides-II* were determined to be sensitive. Only *B.mycooides-I* was determined to be resistant against amicasin antibiotic. The remaining *Bacillus* species were sensitive. The *Bacillus* species were the most resistant to cephalothin among antibiotics such as ceftriaxone, cephalozin and cephalothin. The majority of *Bacillus* species with positive and negative beta-lactamase activity were sensitive to amicasine.

In the research, Iodometric Test and Chromogenic Cephalosporin Test (Nitrocefine) showed the same grade reliability for evaluating the activities of beta-lactamase enzyme of *Bacillus* strains.

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