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Ampicillin resistance profile of local *Bacillus* species isolated from Syrian soils

M. Harba*, M. Jawhar

[1]*Department of Molecular Biology and Biotechnology PO Box 6091 AECS, Damascus. Syria*

Abstract

Antibiotic-resistant bacteria are one of the main global health problems that can affect humans, animals and environment. In this work, out of 525 isolates, 39 *Bacillus* strains, which represented six species as *B. atrophaeus, B. subtilis, Paenibacillus polymyxa, B. amyloliquefaciens* and *B. tequilensis* were *in-vitro* tested for ampicillin resistance on NA media, and profound inhibition of bacteria growth was observed 24 hours post incubation. The data indicated that resistance to ampicillin was species-related as *B. subtilis*, (N = 20; % 50), *B. amyloliquefaciens*, (N = 10; % 60), and *B. atrophaeus* (N = 3; % 33.3), B. *poenibacillus* (N = 2; % 0) and for *B. tequilensis* (N = 4; % 75). The percentage of zone inhibition of the *Bacillus* colonies ranged from 0 to 75 %, compared to the untreated control. However, 51% of tested *Bacillus* strains were not sensitive to ampicillin, suggesting the existence of potential risk for *Bacillus spp.* infections. The results of this study suggest that the Syrian *Bacillus* strains are variable in their susceptibility towards ampicilin, and the presence of resistant strains is of concern about how this resistance could spread to other bacteria, and then to humans.

Keywords: In vitro, ampicillin resistance, Bacillus species, inhibition zone

1.Introduction

The emergence of antimicrobial resistance is one of the biggest public health problem at the 21st century, since it occurs naturally and can be released from the human or animal bodies to river water or soils without degradation [1,2]. The presence of antibiotic-resistant bacteria in soils has been documented throughout the world [3]. However, selection of resistant organisms in nature might result from natural creation of antibiotics by soil organisms, from animals or human's wastes or animal feed or crops runoff [4].

Moreover, although the concentrations of soil antibiotics are usually low in the majority of ecosystems, these concentrations could activate specific bacterial responses that might infect both humans and animals, and the infections are difficult to treat comparing with those caused by non-resistant bacteria [5]. Antibiotic resistance leads to higher healthcare costs, prolonged hospital admissions, and considerably increased mortality [6]. Therefore, knowledge of antimicrobial resistance is still crucial.

Recently, the emergence of antibiotic resistance bacteria has increased and its likely public health consequences have resulted in an enhanced bacterial resistance observation in many countries [5,7]. This concern is emphasized by the fact that Bacilli, in a number of commercially accessible probiotic feed supplements for both humans and animals have been shown to be resistant to different antibiotics [8,9].

Ampicillin (AMP), a semi-synthetic β-lactam antibiotics, is extensively used in human and livestock medicines, but lately its resistance rate has increased [10,11]. It inhibits the synthesis of bacterial cell wall, since the amino group in AMP allows it to penetrate the external membrane of Gram-negative bacteria. It will then become an inhibitor of transpeptidase, which is needed for bacterial cell wall formation, and eventually leads to cell disintegration [12,13].

Although Bacillus spp. are largely used as food microbial supplements, the information available on the antimicrobial susceptibility profiles of *Bacillus*

* Corresponding author: ascientific@aec.org.sy

is quite limited. Even less information is currently available on the AMP susceptibility profiles of *Bacillus spp.* isolated from Syrian soils; therefore, this work was aimed to detect the AMP resistance profile of local *Bacillus spp.* isolated from different soil locations in Syria.

2. Materials and Methods

2.1. Microorganisms

In this work, out of 525 isolates, 39 *Bacillus* species strains comprising 3 *atrophaeus*, *20 subtilis*, *2 P. polymyxa*, *10 amyloliquefaciens* and 4 *tequilensis* were used in this study (Table 1). They were previously isolated from soil samples randomly collected from different regions of Syria [14]. The isolates were grown on nutrient broth (NB) culture, the colonies of prospective *Bacillus* sp. were identified according to Wulff et al. (2002) [15], and the used strains are given in Table 1.

2.2. In vitro evaluation of susceptibility to AMP

To identify susceptibility to AMP, *Bacillus spp*. strains were grown on NB media. susceptibility plates were inoculated and allowed to dry for approximately 5 minutes, before adding the antibiotic disk. After incubating for 24 h at 37°C, the inhibition zones were measured. The culture medium without AMP was used as the control during the whole process. The AMP susceptibility was tested based on the inhibition zone diameter [16], which is classified as susceptible, intermediate and resistant isolates were set as ≥21 mm (susceptible: +), 20-14 mm (intermediate resistant: ++) and >13 mm or no zone of inhibition (resistant: +++). After the incubation period, zone of inhibition appeared around the antibiotic discs. Sensitivity, intermediate resistance and resistances were determined by the zone of complete growth inhibition around each disk.

2.3. Data analysis

All experiments were achieved in triplicat with five Petri dishes per replicate, for each bacterium-AMP *in vitro* evaluation, using a completely randomized design. The presence of a clear zone around *Bacillus* colonies was considered as an index of the AMP sensitivity which were analyzed descriptively and presented here using the diameter of colonies.

Table1. *Bacillus* species used in the study.

***Bacillus* Species**	**Number of strains**	**Morphology**
Atrophaeus	3	Brown-black, opaque, smooth, circular
Amyloliquefaciens	10	Creamy white with irregular margins
Paenibacillus	2	Milky white, thin often with amoeboid spreading
Subtilis	20	white, opaque, rough, with jagged edges Fuzzy
Tequilensis	4	Yellowish, opaque, smooth, circular

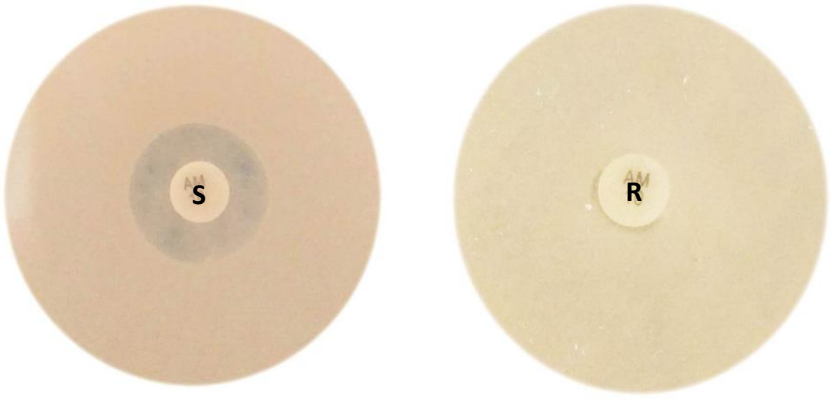

Figure 1. Testing of AMP resistance *in vitro*. These petri dishes contain *Bacillus* (creamy yellow) cultured on NB media. The white discs (in the middle) each contain AMP. Where clear zones appear around the discs, bacterial growth has been prevented by the AMP. R: resistant and S: susceptible.

Table. 2. Ampicillin sensitivity of *Bacillus* species

No.	*Bacillus spp.* Strains	Accession No.	Ampicillin sensitivity
	B.subtilius		
1	SY35A	MT159355	-
2	SY41B	MT159356	++
3	SY44A	MT159357	-
4	SY60A	MT159358	-
5	SY73B	MT159359	-
6	SY113C	MT159360	+++
7	SY116C	MT159361	+++
8	SY118C	MT159362	+++
9	SY124B	MT159363	-
10	SY130D	MT159364	++
11	SY132E	MT159365	-
12	SY133	MT159366	-
13	SY132C	MT159367	-
14	SY134D	MT159368	+++
15	SY135D	MT159369	++
16	SY139D	MT159370	++
17	SY151C	MT159371	++
18	SY160C	MT159372	-
19	SY168C	MT159373	++
20	SY190E	MT159374	-
	B. poenibacillus		
21	SY53C	MT159375	-
22	SY55B	MT159376	-
	B. atrophaeus		
23	SY63E	MT159354	+++
24	SY15B	MT159352	-
25	SY199A	MT159353	-
	B. amyloliquefaciens		
26	SY82C	MT159377	++
27	SY96C	MT159378	-
28	SY96E	MT159379	+
29	SY123A	MT159380	+++
30	SY128B	MT159381	-
31	SY134C	MT159382	-
32	SY159D	MT159383	++
33	SY177C	MT159384	++
34	SY190D	MT159385	-
35	SY200D	MT159386	++
	B. tequilensis		
36	SY69A	MT159387	+++
37	SY145D	MT159388	++
38	SY149C	MT159389	++
39	SY150D	MT159390	-

- (no growth), susceptible (+), intermediate resistant (++) and resistant (+++) according to Masood and Aslam (2010).

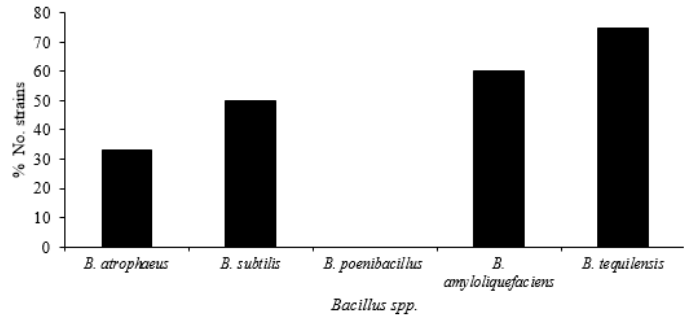

Figure 2. Percentage of *Bacillus* species strains grown under ampicillin treatment

3. Results and Discussion

In the present work, 39 *Bacillus* species including *B. atrophaeus, B. subtilis, P. polymyxa, B. amyloliquefaciens* and *B. tequilensis* were used. Their nucleotide sequences of 16S rRNA gene were deposited at the GenBank under accession numbers MT159352 to MT159390 according to Harba et al. (2020) [17] (Table 2).

The resistance of *Bacillus spp.* to AMP was concluded and validated by the inhibition zone towards AMP on NA media comparing with the control 24 hours post incubation (Fig. 1). The data indicated that susceptibility to AMP was species-related as *B. subtilis*, (N = 20; % 50), *B. amyloliquefaciens*, (N = 10; % 60), and *B. atrophaeus* (N = 3; % 33.3), B. *poenibacillus* (N = 2; % 0) and for *B. tequilensis* (N = 4; % 75) (Table2; Fig. 2). The percentage of zone inhibition of the *Bacillus* colonies ranged from 0 to 75 %, compared with the untreated control. However, 51% of *Bacillus* strains were not sensitive to this AMP, suggesting the existence of potential risk for *Bacillus spp.* infections (Table 2).

Because *Bacillus spp.* have clinical importance, determining their resistance to antibiotics is vital for treatment during outbreaks. Our data showed that *Bacillus spp.* had a kind of resistance towards AMP, and these results are in agreement with several works that reported the resistance of *Bacillus spp.* to multi-antibiotic treatments in several sources, including food [8,18,19].

However, the molecular system of bacterial resistance towards antibiotics is still unclear. Xiang et al. (2001) [20], reported that the resistance level was higher in mutant strains than that of wild type strains, and there was a quantitative reaction between point mutations and bacterial resistance levels.

Moreover, it has been found that the antibiotic resistance of bacterial strains results generally from either chromosome mutations or acquisition of resistance plasmids [21], therefore, the use antibiotics by human should be strongly regulated to reduce the chance of microorganisms to build up resistance.

It has been reported that AMP has a β-lactam ring that inhibits penicillin-binding proteins (PBPs), and during the PBPs interact with β-lactam rings, these proteins can't catalyze the synthesis of new peptidoglycan, disrupting the formation of the cell wall of bacteria. Therefore, the AMP resistant bacteria produce β-lactamase enzyme for cleaving the β-lactam ring of AMP to inhibit it. Several cloning vectors containing a resistance *bla* gene, produce β-lactamase enzyme [22, 23,24].

Li et al. [24] suggested that different Genes *frdD, ftsI, acrB, OmpD, marR, VgrG,* and *envZ* are associated with AMP resistance. Studies have demonstrated that the *frd* gene encodes a FRD enzyme to catalyze the conversion between fumarate reductase and succinate dehydrogenase, it is considered that the *frdD* gene is involved in certain metabolic pathways, maybe linked with AMP resistance [24, 25]. It is also reported that ABC (ATP binding cassette) efflux transporters of *B. subtilis* can generate tolerance to lincosamide, which is consistent with our results [19].

4. Conclusion

In the present study, the AMP resistance profiles of the isolated soil *Bacillus spp.* were identified on NA media. Results showed that 51% of *Bacillus* strains were not sensitive to ampicillin, suggesting the existence of potential risk for *Bacillus spp.* infections. Therefore, antimicrobial susceptibility testing is required as routine microbiological analyses of soils in Syria.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest.

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