18th International Conference on Environmental Science and Technology

Athens, Greece, 30 August to 2 September 2023

Antibiotic Susceptibility of *Erwinia amylovora* Isolates from Fire Blight Diseased Pomaceous Fruit Trees in Georgia

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Abstract Fire blight, caused by the bacterial pathogen, *Erwinia amylovora* is a devastating disease of fruit trees belonging to the *Rosaceae* family. The most susceptible species are apple, pear and quince. The economic loss caused by bacterial blight of fruit trees is manifested both in crop loss and rapid destruction of entire orchards. It is difficult to control fire blight as the *E. amylovora* has significant survival capacity. The goal is to study the antibiotic susceptibility of the *E. amylovora* Georgian isolates. Dozens of the pathogen isolates were obtained and identified from the fire blight diseased apple, pear and quince trees in eastern Georgia. Susceptibility of these isolates towards eight antibiotics: streptomycin, penicillin, tobramycin, erythromycin, kanamycin, ofloxacin, trimethoprim and tetracycline by disc-diffusion method have been studied. The majority of *E. amylovora* isolates from different regions of eastern Georgia are sensitive to tobramycin, kanamycin, ofloxacin, tetracycline and streptomycin, with the exception of isolates ## 6052, 6053 8892, which express some resistance to streptomycin. The absolute majority of the *Erwinia amylovora* isolates are resistant to penicillin and trimethoprim, while the susceptibility to erythromycin is variable.
Keywords: Fire blight, *Erwinia amylovora*, antibiotic susceptibility

1. Introduction

Erwinia amylovora is a devastating bacterial pathogen that causes the destructive fire blight disease of plants of *Rosacease* family. The disease is causing considerable damage and economic losses in apple (*Malus domestica*), pear (*Pyrus communis*) and also in quince (*Cydonia oblonga*) trees. The disease enters the tree at the tips of the branches and then **translocate** down the stems causing dieback.
Fire blight symptoms of fire blight are seen on blossoms, leaves, shoots, fruits branches and trunk. Affected organs wilt rapidly and become brown or black. Died leaves are remained on the tree. Reddish water soaked lesions develop on the bark oozing an orange-brown liquid. The diseased branches or the whole plant looks like burned (Vanneste 2000).
Oozing bacteria are spread by wind, rain and insects and infect new plants.
A small number of the bacteria overwinter at the margins of branch and trunk cankers and the disease cycle will start the following spring about blossoming time.

The indigenous to North America fire blight disease (1870) now occurs in countries around the world including countries in North America, Europe, North Africa, Middle East, Oceania and Asia (EPPO 2017). It reached Georgia in the 2016 (Gaganidze et al., 2018) and spread to some regions of the country (Gaganidze et al., 2021).

Control of fire blight is difficult because currently there are no available synthetic compounds that directly affect the pathogen. Since 1950, antibiotics have been used in agriculture against bacterial diseases (McManus et al., 2002). Antibiotics are essential for control of bacterial diseases of plants, especially fire blight of pear and apple (Strockwell and Duffy, 2012). Antibiotics are applied on leaves and flowers to supress growth of phytopathogens before infection

The most commonly used on plants antibiotics are oxytetracycline and streptomycin. Streptomycin spraying is the most effective control procedure to prevent infection during booming but is not allowed in most countries due to occurrence of resistance of strains of *E. amylovora* to this antibiotic (Chiou and Jones, 1993; McGhee and Sundin, 2011).

Despite this, when the various chemical and biological treatments fail to keep the fire blight infection at an acceptable level in years of heavy disease pressure the Swiss authorities allow the controlled use of the antibiotic streptomycin (Gusberty et al., 2015).

This paper demonstrates the results of the study of *E. amylovora* Georgian isolates susceptibility to different antibiotics by disc diffusion method.

2. Materials and Methods

Bacterial strains were isolated from the diseased apple, pear and quince tree samples, collected in the eastern Georgia in 2021-2022. After purification, bacterial isolates were identified as *E. amylovora* by conventional

laboratory methods, including ELISA test and by PCR using the primer pairs A/B (Bereswill et al., 1992) and G1-F/G2-R (Taylor et al., 2001) were used in the study.

The disk diffusion method was used to determine bacterial susceptibility against antibiotics EUCAST "Antimicrobial susceptibility testing: EUCAST disk diffusion method"). Overnight cultures of *Erwinia amylovora* isolates from the LB broth were plated on a solid NSA plate, after a 5 minute delay the excess fluid was decanted and commercial antibiotic discs were placed on them with the use of sterilized forceps: tobramycin (10 µg/ml), penicillin (1IU), trimethoprim (5µg/ml), erythromycin (15µg/ml), kanamycin (30 µg/ml), streptomycin (10 µg/ml), ofloxacin (5 µg/ml), tetracyclin (30 µg/ml). The plates were incubated at 25°C for 48 h in the thermostat. At the end of the incubation, the diameters of the clear zones around the antibiotic discs were measured. The studied isolates were classified as follows: sensitive - S (inhibition zone diameter ≥ 14 mm), intermediate - I (inhibition zone diameter 11-13 mm) and resistant - R (inhibition zone diameter ≤ 10 mm (Kenny et al., 1992).

3. Results and discussion

Antibiotic susceptibility of *E. amylovora* Georgian isolates is represented in Table and Figure.

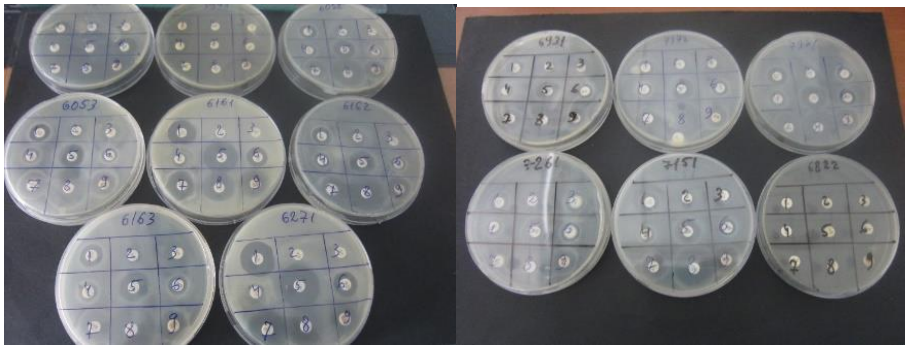

Figure 1. Antibiograms of *Erwinia amylovora* isolates obtained from fire blight diseased plants from different regions of Georgia. Left: Kakheti, Sighnaghi municipality, village Jugaani, isolates ##: 5833, 5941, 6052, 6053, 6161, 6162, 6163, 6271.Right: Shida Kartli, Khashuri municipality, village Gomi, isolates ##: 6931, 7372, 7371,7261, 7151, 6822.

Table. Antibiotic susceptibility of *Erwinia amylovora* Georgian isolates

***E.amilovora* isolate N**	**Place of isolation**	**Zone of inhibition, mm**							
		Antibiotic							
		Tobramycin	**Penicillin**	**Trimethoprim**	**Erythromycin**	**Kanamycin**	**Streptomycin**	**Ofloxacin**	**Tetracycline**
5831	Kakheti, Tsnori, Jugaani (apple)	18 S	17 S	8 R	24 S	23 S	19 S	23 S	23 S
5833		21 S	8 R	8 R	22 S	23 S	23 S	22 S	23 S
6052		17 S	8 R	8 R	18 S	19 S	8 R	22 S	22 S
6053		17 S	8 R	8 R	8 R	18 S	8 R	21 S	21 S
6161		22 S	8 R	8 R	8 R	21 S	17 S	22 S	22 S
6163		16 S	8 R	8 R	12 I	19 S	12 I	19 S	22 S
6271		22 S	8 R	12 I	8 R	23 S	16 S	23 S	23 S
7591	Shida Kartli, Khashuri, Gomi (pear)	21 S	8 R	8 R	22 S	23 S	18 S	23 S	22 S
7610		19 S	8 R	18 S	12 I	22 S	18 S	22 S	22 S
6931	Shida Kartli, Khashuri, Gomi (quince)	18 S	8 R	8 R	18 S	22 S	18 S	22 S	21 S
7371	Shida Kartli, Khashuri, Gomi, (apple)	21 S	12 I	12 I	12 I	22 S	18 S	22 S	22 S
8892	Shida Kartli, Gori, Karaleti	22 S	20 S	8 R	8 R	23 S	8 R	22 S	22 S
8121		22 S	8 R	8 R	8 R	22 S	21 S	12 I	16 S

*S -susceptible (inhibition zone diameter ≥14 mm), I- intermediate (inhibition zone diameter 11-13 mm), R-resistant (inhibition zone diameter ≤10 mm).

The results indicate that the majority of *E. amylovora* isolates ## 5833, 6052, 6053, 6163 (Kakheti, Sighnaghi municipality, village Jugaani), ## 6821, 6822, 6931, 7591 (Shida Kartli, Khashuri municipality, village Gomi), # 8121 (Shida Kartli, Gori municipality, village Karaleti) are resistant (R) to penicillin and to trimethoprim. Isolates ## 6053, 6271, 8121, and 8892 are also resistant to erythromycin.

Almostall isolates are susceptible to tobramycin, kanamycin, ofloxacin and tetracycline.

Different picture is obtained for the isolate # 7371 from apple collected in Shida Kartli, Khashuri, Gomi, which is sensitive to most of the tested antibiotics and intermediate to the rest – penicillin, trimethoprim and erythromycin.

As for the streptomycin, all studied isolates are susceptible to streptomycin, except isolates ## 6052, 6053, 8892, which are resistant.

Different susceptibility to antibiotics has been demonstrated for several *E. amylovora* isolates (Spitko, Alvarado, 1999; Donat et al., 2004; Islam et al., 2014). *E. amylovora* Croatian isolates were susceptible to streptomycin and other antibiotics tested. Authors explain this by the fact that no antibiotics are allowed in plant disease control in Croatia (Dermic et al. 2006). Based on special research of the agricultural formulations the likelihood for one potential factor in resistance development due to streptomycin use is diminished (Rezzonico et al., 2009).
Erwinia amylovora can develop resistance to streptomycin through a single nucleotide mutation in the *rpsL* gene resulting in amino acid substitution
that prevents inhibitory binding of streptomycin while preserving the functionality of the ribosome (McManus et al., 2002). Recently, two other *rpsL* mutations were identified in resistant colonies of *E. amylovora* resulting in conditional-lethal streptomycin-dependent (Sm^D) phenotypes, which are unable to grow in absence of the antibiotic (Escrusell et al., 2020).

Some aspects of antimicrobial drug resistance and its emergence have been elucidated. Inactivation of antimicrobials occurs by some common mechanism such as drug inactivation/degradation by bacterial enzymes or alteration in the bacterial targets or expulsion of drug out of the bacterial cells (Kumar and Singh, 2013).

Study of the mechanism of revealed resistance of some *E. amylovora* Georgian isolates to streptomycin is being carried out.

Conclusions:

The majority of *E.amylovora* isolates from different regions of eastern Georgia are sensitive to tobramycin, kanamycin, ofloxacin, tetracycline and streptomycin, with the exception of isolates ## 6052, 6053 and 8892, which express resistance to streptomycin. The absolute majority of the *E.amylovora* isolates are resistant to penicillin and trimethoprim, while the susceptibility to erythromycin is variable.

Acknowledgements:

This work is funded by the Shota Rustaveli National Science Foundation of Georgia (SRNSFG), grant number FR-19-22524. The participation of Dr. Fabio Rezzonico to this work was funded under project number IZ08Z0_177515 by the r4d Programme of the Swiss Agency for Development and Cooperation (SDC) and the Swiss National Science Foundation (SNSF).

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