

Antibiotic Sensitivity of Some Phytopathogen Bacteria of Genus *Pseudomonas* Isolated from Fruit Trees of Azerbaijan

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Abstract: Two hundred and seventy five (275) samples were taken from the plants of pear, blackberry, cherry, mandarin, walnuts and grape grown in Azerbaijan and 142 bacterial strains belonging to the genus *Pseudomonas* were isolated. All bacterial strains showed hypersensitivity against antibiotics amoxicillin, ampicillin and cefazolinom and no resistant strain was noticed against these antibiotics. All the strains of *Ps. cerasi*, *Ps. citri*, *Ps. piri*, *Ps. rubri* and *Ps. vitivorum* showed hypersensitivity against antibiotics ceftazidime, cefazolinum and moxifloxacin and no full-resistant strain was detected against these antibiotics. The strains of *Pseudomonas rubri* and *Ps. vitivorum* demonstrated hypersensitivity against ceftriaxone and amikacine, respectively, with no resistant strain against these antibiotics. *Pseudomonas cereasi* and *Ps. citri* strains showed high resistance against cefoxitin antibiotics, while *Ps. juglandis* strains depicted resistance against cefuroxime antibiotics, *Ps. rubri* strains against azithromycin antibiotics.

Keywords: Antibiotics, phytopathogen bacteria, *Pseudomonas*, sensitivity

1. INTRODUCTION

At present about 300 bacterial species cause plants diseases [3]. These bacteria are mainly belong to the species of *Agrobacterium*, *Bacillus*, *Corynebacterium*, *Erwinia*, *Mycobacterium*, *Pectobacterium*, *Pseudomonas* and *Xanthomonas* [7,10].

When phytopathogen bacteria infect plant tissue, firstly they degrade intercellular matter (pectin), as a result, of which, the infected tissue softens and rots. Such bacterial rot is usually observed in a bulb- and root-crops and plant stem. Rotting plant body is exposed to the attack of other bacteria and fungi that stimulate rot [5,7].

The genus *Pseudomonas* comprizes of the most damaging species of bacteria in plants. More than 50 species of this genus are known to induce various diseases. However, some species specifically cause the diseases in some specific plants. To develop an effective management strategy against such diseases, it is essential to evaluate the effects of antibiotics against a specific pathogen both under in vitro and in vivo conditions [6,11].

Antibiotic sensitivity of phytopatogenic bacteria *Pseudomonas beticola*, *Ps.campestris*, *Ps. carotue*, *Ps. holei*, *Ps. maculicolum*, *Ps. solanacearum*, *Ps. tumefaciens* and *Ps. vesiatonia* isolated from maize, beetroot, cabbage, potatoes, tomato and carrot in Azerbaijan [8,9].

The present investigation was aimed to study the antibiotic sensitivity of genus *Pseudomonas* isolated from the plants in Azerbaijan.

2. MATERIALS AND METHODS

A total of 250 samples have been taken from the diseased plants of pear, blackberry, cherry, mandarin, walnuts, grape from different regions (Absheron, Astara, Balakan, Gabala, Guba, Gusar, Goychay, Ismailli, Lankaran and Khachmas districts) and 142 strains isolated in pure form during the years of 2014-2016 in the territory of Azerbaijan. Leaves and fruits of plant were collected in sterile packets. At each time of collection, precaution was taken to minimize cross contamination of samples. In order to isolate bacteria and produce pure cultures synthetic nutrient-agar medium mixed with yeast

extract was used [9]. Medium was prepared by dissolving glucose-20,0 g, pepton-3,0 g, yeast extract-3,0 g, p- aminobensoic acid-0,05 g, NaCl-0.3 g, ađar-20 g into 1l of distilled water. The snips (1,5-2,0 sm) were taken from plant samples, put on surface of solid medium in Petri-dish and incubated at 35⁰C. Derived cultures were kept in a fridge at 4⁰C till use.

The identification of bacterial strains was done as per to *Bergey's manual of systematic Bacteriology* [2] on the basis of morphology (Gram's staining was performed to determine the size, shape, arrangement and Gram reaction of the izolates), physiological (growth at diferent temperature and at 4% NaCl), biochemical signs (Oxidative-Fermentative test, Nitrate Reduction test, Citrate Utilization test, Urease test, Sucrose fermentation test, Triple Sugar Iodine test, Mannitol Fermentation test, Gelatine Hydrolysis) were done [4].

The sensitivity of the bacteria strains against a new class of antibiotics under *in vitro* conditions was studied by standard agar disk-diffusion method [1].

For this, the standard (ready) disks (Liofilchem, Roseto dađlı Abeuzzi, Italian) with absorbed antibiotics of *amoxicillin*, *ampicillin*, *azithromycin*, *moxifloxacin*, and *cefotaxime*, *ceftazidime*, *cefriaxone*, *cefuroxime*, *cefazolinom*, *cefoxitin*, *ciprofloxacin* *cefalexinom* have been used.

The 10 mkl of 0,5 Mac-Farland suspension of the fresh (24 h) bacterial culture was smeared on the surface of nutrient agar and kept for 30 min in a thermostat. Then the standart disks with corresponding antibiotics were put on the surface of nutrient ađar (in Petri plates) and incubated for 24-48 h at 35⁰C. An intact transparent zone around the disk considered as the full sensitivity to antibiotics; while the variant at the end of some colonies in transparent zone was taken as middle sensitivity.

3. RESULTS AND DISCUSSION

One hundred and forty two bacterial strains belonging to genus *Pseudomonas* were isolated from 275 plant samples. Of these 32 strains from pear tree were identified as *Ps. piri*, 26 isolates from blackberry as *Ps. rubri*, 20 isolates from cherry as *Ps. cerasi*, 39 isolates from mandarin as *Ps. vitivorum*, 11 isolates from walnut as *Ps. juglandis*. 78-100 % strains of *Ps. cerasi* showed the sensitivity against antibiotics *amoxicillin*, *ampicillin*, *moxifloxacin*, *ceftazidime* and *cefazolinom* while none was resistant. However, 84% strains were resistant against *cefoxitin*, 46% and 56% strains- to *azithromycin* and *cefuroxime*, 40% strains-to *cefotaxime* and *cefalexinom*, 20, 11 and 4% strains accordingly-against antibiotics of *ciprofloxacin*, *amikacin* and *ceftriaxon*. No any strain sensitivity against *cefoxitin* was observed (Table 1).

The sensitivity of *Ps. citri* strains against antibiotics was alike with that of *Ps. cerasi* (Table 1). So that, 86-100% strains indicated the sensitivity against the antibiotics of *amoxicillin*, *ampicillin*, *moxifloxacin*, *ceftazidime* and *cefazolinom*. No resistant strains against these antibiotics were observed.

Table1. Antibiotic sensitivity of strains of bacteria *Ps. cerasi* and *Ps. citri*

Antibiotics	Number of strains, %					
	<i>Ps.cerasi</i>			<i>Ps.citri</i>		
	Hyper sensible	Medium sensible	Resistant	Hyper sensible	Medium sensible	Resistant
<i>Amikacin</i>	63	26	11	54	32	14
<i>Amoxicillin</i>	100	0	0	94	6	0
<i>Ampicillin</i>	98	2	0	96	4	0
<i>Azithromycin</i>	32	22	46	38	21	41
<i>Moxifloxacin</i>	100	0	0	100	0	0
<i>Cefotaxime</i>	24	36	40	32	24	44
<i>Ceftazidime</i>	94	6	0	88	12	0
<i>Ceftriaxone</i>	82	14	4	77	18	5
<i>Cefuroxime</i>	38	6	56	30	12	58
<i>Cefazolinom</i>	78	22	0	86	14	0
<i>Cefoxitin</i>	0	16	84	0	8	92
<i>Ciprofloxacin</i>	48	32	20	51	30	19
<i>Cefalexinom</i>	44	16	40	46	16	38

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There were not resistant strains of *Ps. juglandis* species against the antibiotics of *amoxicillin*, *ampicillin* and *cefazolinom*. The strain with full sensitivity against

cefuroxime was not observed. 74% strains were resistant against these antibiotics. 56 % strains indicated the resistance against the antibiotics of *azithromycin*, *cefalexinom* and *moxifloxacin* and 50-62 % strains - against *cefoxitin*, *ceftriaxone* and *cefotaxime*. The amount of sensitive and resistant strains were equal against the antibiotics of *amikacin* and *ceftazidime* (Table 2).

Antibiotic sensitivity of the strains of *Ps. piri* is somewhat similar with those of *Ps. cerasi* and *Ps. citri* strains. So that, 88-100 % strains have shown the sensitivity against the antibiotics of *amoxicillin*, *ampicillin*, *moxifloxacin*, *ceftazidime* and *cefazolinom* and there have not been any resistant strains against these antibiotics (Table 2). Nevertheless, according to correlations with antibiotics of *cefotaxime*, *ceftriaxone*, *cefuroxime* and *cefoxitin* the strains of this species have been different from strains of *Ps. citri* and *Ps. cerasi* species and 38-52% of *Ps. piri* strains showed the sensitivity against these antibiotics.

78-100 % strains of *Ps. rubri* showed the sensitivity against the antibiotics of *amoxicillin*, *ampicillin*, *moxifloxacin*, *ceftazidime*, *ceftriaxone* and *cefazolinom* and there have not been any resistant strains against these antibiotics). The full- sensitive strains have not been observed against *azithromycin* antibiotic and the amount of resistant strains against this antibiotic was 70%. 47-54% strains were

Table2. Antibiotic sensitivity of strains of *Ps. juglandis* and *Ps. piri*

Antibiotics	Number of strains, %					
	<i>Ps.juglandis</i>			<i>Ps.piri</i>		
	Hyper sensible	Medium sensible	Resistant	Hyper sensible	Medium sensible	Resistant
<i>Amikacin</i>	43	12	45	45	42	13
<i>Amoxicillin</i>	100	0	0	94	6	0
<i>Ampicillin</i>	100	0	0	88	12	0
<i>Azithromycin</i>	18	26	56	24	33	43
<i>Moxifloxacin</i>	24	20	56	100	0	0
<i>Cefotaxime</i>	22	16	62	52	16	32
<i>Ceftazidime</i>	42	16	42	94	6	0
<i>Ceftriaxone</i>	35	5	60	38	12	50
<i>Cefuroxime</i>	0	26	74	52	6	42
<i>Cefazolinom</i>	88	12	0	90	10	0
<i>Cefoxitin</i>	28	38	50	49	21	30
<i>Ciprofloxacin</i>	66	23	19	56	22	22
<i>Cefalexinom</i>	21	24	56	44	22	34

resistant against the antibiotics of *cefoxitin*, *amikacin*, *cefotaxime* and *cefuroxime*. The amount of sensitive and resistant strains was approximately the same against antibiotics of *ciprofloxacin* and *cefalexinom* (Table 3).

Table3. Antibiotic sensitivity of strains of bacteria *Ps. rubri* and *Ps. vitivorum*

Antibiotics	Number of strains, %					
	<i>Ps.rubri</i>			<i>Ps.vitivorum</i>		
	Hyper sensible	Medium sensible	Resistant	Hyper sensible	Medium sensible	Resistant
<i>Amikacin</i>	23	28	49	57	43	0
<i>Amoxicillin</i>	96	4	0	90	10	0
<i>Ampicillin</i>	100	0	0	95	5	0
<i>Azithromycin</i>	0	30	70	32	34	34
<i>Moxifloxacin</i>	96	4	0	98	2	0
<i>Cefotaxime</i>	22	24	54	48	6	46
<i>Ceftazidime</i>	88	12	0	58	42	0
<i>Ceftriaxone</i>	80	20	0	56	6	38
<i>Cefuroxime</i>	38	8	54	46	3	51
<i>Cefazolinom</i>	78	22	0	82	18	0
<i>Cefoxitin</i>	27	26	47	21	30	49
<i>Ciprofloxacin</i>	44	23	43	60	23	17
<i>Cefalexinom</i>	38	26	36	43	12	45

57-98% strains of *Ps. vitivorum* showed the sensitivity against the antibiotics of *amikacin*, *amoxicillin*, *ampicillin*, *ceftazidime*, *cefazolinom* and *moxifloxacin* and there have not been any resistant strains against these antibiotics. The number of the sensitive and resistant strains against the antibiotics of *azithromycin*, *cefotaxime*, *cefuroxime* and *cefalexinom* was very close. 51% and 49% strains were resistant accordingly against *cefuroxime* and *cefloxitin* antibiotics.

4. CONCLUSION

So, it was determined that all the investigated bacterial strains showed high sensitivity against *amoxicillin*, *ampicillin* and *cefazolinom* antibiotics and resistant strains were not observed against these antibiotics. All the strains of *Ps. cerasi*, *Ps.citri*, *Ps. piri*, *Ps. rubri* and *Ps. vitivorum* species demonstrated the hypersensitivity against *moxifloxacin* and *ceftazidime* antibiotics. At the same time, the strains of *Ps. rubri* demonstrated the hypersensitivity to *ceftriaxone* antibiotic and the strains of *Ps. vitivorum* did the same to *amikacin* antibiotic and their resistant strains to these antibiotics were not observed. There were high resistant strains of *Ps. cerasi* and *Ps. citri* to *cefloxitin* antibiotic, *Ps. juglandis* - to *cefuroxime* antibiotic and *Ps. rubri* - to *azithromycin* antibiotic.

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