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Occurrence of endophytic *Escherichia coli* strains with antibiotic resistance in fresh beetroots of different traders

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Abstract:
Background*:Fresh vegetables and fruits are one of the most important habitats for endophytic bacterial communities. These communities may contain pathogenic and opportunistic microorganisms such as E. coli, Listeria monocytogenes, Salmonella enterica, Pseudomonas spp., etc. Endophytic strains of these species may be resistant to various antibiotics. In our work, we examined the internal tissues of beetroots of different traders (Kyrgyzstan, Turkey, Russia) for the presence of E. coli and evaluation of resistant strains against widely used antibiotics.*
Materials and Methods*:Beetroots were purchased from retail networks. A total of 69 vegetables were analyzed. The traditional plating method on a highly selective chromogenic medium REBECCA® EB was used to study the community of endophytic E. coli. Three colonies from beets of different origins were isolated in a pure culture andidentified based on 16S rDNA nucleotide sequence data using the BLAST NCBI. Antibiotic susceptibility of the E. coli strains was tested using Mueller-Hinton agar. Disks were tested with a wide range of antimicrobial drugs:Amoxicillin 10 (µg/disk) (AMO), Ampicillin 10 (µg/disk) (AMP), Meropenem 10 (µg/disk) (MER), Pefloxacin 5(µg/disk) (PEF), Streptomycin 300 (µg/disk) (STR), Ticarcillin+clavulanic acid 75 (µg/disk) (TIC), Fosfomycin 200 (µg/disk) (FOS), Ceftibuten 30 (µg/disk) (CEF), Ciprofloxacin 10 (µg/disk) (CIP). A total of 124 strains of E. coliweretested.*
Results*: Endophytic E. coli were detected in 56% of beet samples from different traders. The percentage of strains resistant to at least one of the widely used antibiotics tested was 15%. Most resistant E. coli strains were isolated from Turkeybeets.*
Conclusion:*Thus, consumption of fresh beet may pose some risk to public health due to the presence of antibiotic-resistant strains of E. coli.*

Key Word*:**beet; fresh vegetables; Escherichia coli; endophytic microorganisms; antibiotic-resistance*.

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I. Introduction

Endophytic microorganisms are one of the most promising areas in the study of microbe-agricultural plant associations. First of all, because endophytic microorganisms are able to synthesize various plant growth-promoting factors such as phytohormones (auxins, zeatin), etc[1,2,3]. The strains that produce important phytohormones are often responsible for microbial stimulation of germination, growth and development of higher plants [4,5]. Microbial synthesis of phytohormones also contributes to the suppression of growth of some phytopathogenic microorganisms [6,7,8]. But not all endophytic microorganisms can promote plant growth, improve nitrogen supply, etc. In some cases, they are human pathogens and opportunistic species [9,10]. The continued increase in the number of foodborne illnesses associated with foods such as fresh fruits and vegetables challenges the notion that opportunistic and pathogenic microorganisms are defined primarily by their ability to colonize the intestinal habitat [9,11,12,13]. It has been reported that raw vegetables in particular harbor potential foodborne pathogens, including *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas*spp, *Salmonella enterica*, *Candida parapsilosis*, etc[9,10,14,15,16]. Certainly, vegetables provide beneficial nutrients to the human body such as vitamins, fiber, and ash. However, if these vegetables are contaminated, they could be a source of infection. They may pose a potential threat to human health, especially to immunocompromised individuals. Another problem is that endophytic strains of opportunistic and pathogenic microorganisms exhibit resistance to widely used antibiotics, which have become a significant clinical problem [17,18,19,20]. Among the most studied and popular vegetables worldwide in which endophytic pathogenic *Enterobacteriaceae*, particularly *E. coli*, have been repeatedly detected are cabbage, celery, lettuce, parsley, leeks, radish, basil, and

spinach [10,14,15,20]. Beetroot is not one of them. Nevertheless, this vegetable is used not only in sugar production and other industries. Beet has also attracted much attention as a health-promoting and disease-preventing functional food [21]. The aim of our work was to examine the internal tissues of beets from different traders (Kyrgyzstan, Turkey, Russia) for the presence of *E. coli* and to evaluate the resistance of the isolated strains to common antibiotics.

II. Material And Methods

Study location and sampling

Beets were purchased from trade networks in the Moscow region (imports from Kyrgyzstan, Turkey, and supplies from Russia). A total of 69 vegetables were analyzed (23 beetroots of different origins each).

Microbiological analyses and species identification

To study endophytic *E coli* communities, vegetables were treated according to the following scheme: 70% ethanol, 30 min; 2% sodium hypochlorite, 30 min; 70% ethanol, 30 s; and washing in sterile distilled water for 10 min[17,18]. After the exocarp was removed with a sterile scalpel, the internal tissues were cut out, homogenized, and poured with sterile water to obtain 1:10, 1:100 and 1:1000 dilutions.

To desorb the bacteria from tissues, water suspensions were treated by ultrasound in the device UDZN (22 kHz, 0.44A, 2 min). The prepared suspensions were plated in six replicates each on REBECCA® EB (media for direct next-day enumeration of *E. coli*, bioMerieux, Inc., France), a highly selective chromogenic medium (NF Validation EN ISO 16140). Plates were incubated at 37 °C for 26 hours.

Three colonies from beet of different origin were isolated into a pure culture and identified based on 16S rDNA nucleotide sequence data using the BLAST NCBI. DNA isolation from pure bacterial cultures was performed using the PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, USA) kit according to the manufacturer's recommendations. Sequencing of the PCR product of the variable sequence v3-v4 of the 16S rRNA region was performed according to the standard protocol of the MicroSeq 500 16S rDNA Bacterial Identification Kit (MicroSEQ™ 500 16S rDNA Identification User Guide, Thermo Fisher, USA) using standard fD1/rD1 primers. DNA sequencing was performed using an ABI Prism 3130 genetic analyzer. To analyze the obtained electrophoretograms and nucleotide sequences, we used MicroSeq ID v.2.0 (Applied Biosystems, USA) software and the validated MicroSeq ID 16S rDNA 500 Library v2.0 (Table no 1).

Table no 1. Identification of endophytic Escherichia coli strains from different traders.

Origin	Sequenceprocessed
Turkey	>SB1_537r_
	GGCGTGTCTCGTCTGCGGGTACGCAGGTGCAGCCTAGAGTATTAACTTTACTCCCTTCCT
	CCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATC
	AGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTG
	TCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAAACCAGCTAGGGATCGTCGCCTAGGTGA
	GCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGGCCCGAA
	GGTCCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATC
	CCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGCAAAAAA
	GCAAGCTTCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAAT
	CTGAGCCAGATCAAAAACTATA
Kyrgyzstan	
	>SB2_537r_
	ACGAGGTCTCTCTGCGGGTACGTCAATGAGCAAAGGTATTAACTTTACTCCCTTCCTCCC
	CGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGG
	CTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCT
	CAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCC
	GTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGGCCCGAAGGT
	CCCCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCC
	CTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGCAAAAAAGCA
	AGCTTCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTG

Russia	AGCCAGGTTTAAAAACTCTA
	>SB3_537r_
	GAGGGGCTCTTCTGCGGGTACGTCATGAGCAAGGTATTAACTTTACTCCCTTCCTCCCCG
	CTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCT
	TGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCA
	GTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGT
	TACCCCACCTACTAGCTAATCCCATCTGGGCACATCCGATGGCAAGAGGCCCGAAGGTCC
	CCCTCTTTGGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCT
	CCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCACTCGTCAGCAAAAAAGCAAG
	CTTCTTCCTGTTACCGTTCGACTTGCATGTGTTAGGCCTGCCGCCAGCGTTCAATCTGAG
	CCAGAAAAAAAAAAATCTAA

Antibiotic sensitivity of *Escherichia coli* strains

Antibiotic susceptibility of *E. coli* strains was tested using Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd., India) which is a standard medium for disk diffusion method (Bauer-Kirby test) as per the guidelines of Global Laboratory Standards for a Healthier World (CLSI) of USA. Disks were tested with a wide range of antimicrobial drugs (HiMedia Laboratories Pvt. Ltd., India): Amoxicillin 10 (µg/disk) (AMO), Ampicillin 10 (µg/disk) (AMP), Meropenem 10 (µg/disk) (MER), Pefloxacin 5 (µg/disk) (PEF), Streptomycin 300 (µg/disk) (STR), Ticarcillin+clavulanic acid 75 (µg/disk) (TIC), Fosfomycin 200 (µg/disk) (FOS), Ceftibuten 30 (µg/disk) (CEF), Ciprofloxacin 10 (µg/disk) (CIP). As a control, the reference strain of *E. coli* ATCC 2592 was used, which is recommended by the CLSI (https://clsi.org). A total of 124 strains of *E. coli* were tested. Each of the strains was tested in three plate replicates for each of the 9 antimicrobial drugs.

Statistical analysis

The statistical processing of the results was performed using the STATISTICA 8 software(StatSoft, USA).

III. Result

E. coli was isolated from 56% of all beet samples analyzed. The abundance of *E. coli* in the internal tissues of beets from different traders differed only slightly and was 81.2%, 78.4%, and 76.5% in samples from Turkey, Russia, and Kyrgyzstan, respectively. Of all strains isolated and tested for antibiotic resistance by the disk diffusion method, the percentage of those resistant to at least one of the antibiotics tested was 15%. Most resistant strains were isolated from beet samples from Turkey (46%); from samples from Russia and Kyrgyzstan, 29% and 25%, respectively.(Table no 2).

Table no 2. Strains of endophytic *Escherichia coli* isolated from beetroots from retail with antibiotic susceptibility (retarded growth with standard deviations, mm) below the reference values**.

Strain	Origin	AMO***	AMP	MER	PEF	STR	TIC	FOS	CEF	CIP
ECT11	Turkey	17.0±0.06	16.0±0.09	13.3±0.06	20.0±0.38	24.0±0.07	25.7±0.03	25.7±0.09	19.3±0.09	15.8±0.09
ECT16	Turkey	14,0±0,06	14.0±0.07	15.3±0.03	15.0±0.12	6.0±0.07	16.0±0.13	26.7±0.03	19.0±0.07	22.7±0.18
ECT34	Turkey	16.0±0.03	16.7±0.03	14.7±0.07	17.0±0.12	18.3±0.15	19.3±0.09	21.0±0.10	19.3±0.03	23.3±0.09
ECT38	Turkey	16.7±0.07	14.0±0.03	15.3±0.03	18.7±0.07	10.0±0.13	15.0±0.07	20.3±0.07	19.0±0,07	17.3±0.15
ECT41	Turkey	16.0±0.06	15.7±0.06	16.0±0.07	12.7±0.09	16.3±0.06	9.7±0.09	20.3±0.03	9.3±0.06	21.0±0.10
ECT43	Turkey	13.9±0.03	15.0±0.07	17.3±0.07	18.0±0.18	18.3±0.03	23.7±0.03	16.0±0.07	22.0±0.07	25.3±0.12
ECT45	Turkey	22.0±0.03	16.3±0.09	15.3±0,09	17.0±0.07	11.0±0.12	21.7±0.10	23.7±0.06	20.3±0.18	22.3±0.09
ECT48	Turkey	17.1±0.03	13.6±0.06	14.2±0,06	20.2±0.12	14.2±0.07	23.4±0.12	12.6±0.06	20.5±0.13	20.4±0.07
ECT49	Turkey	22.0±0.09	18.0±0.12	16.0±0,07	20.7±0.20	14.3±0.15	21.9±0.09	22.7±0.03	21.3±0.06	16.3±0.09
ECR9	Russia	11.0±0.18	16.3±0.09	15.3±0.03	12.0±0.23	17.3±0.07	16.0±0.12	27.7±0.07	13.7±0.09	23.0±0.07
ECR11	Russia	13.9±0.03	16.3±0.15	16.1±0.03	21.4±0.17	12.8±0.07	22.8±0.07	22.8±0.07	19.2±0.10	18.2±0.07
ECR18	Russia	12.3±0.07	19.0±0.15	14.3±0.07	13.3±0.06	16.3±0.10	21.7±0.09	24.0±0.07	11.7±0.15	23.0±0.15
ECR21	Russia	14.2±0.03	16.2±0.15	16.1±0.03	15.8±0.24	12.4±0.20	18.2±0.23	15.2±0.12	22.5±0.15	16.8±0.03
ECR29	Russia	15.1±0.09	15.8±0.25	16.0±0.07	17.4±0.03	12.2±0.18	17.5±0.03	16.8±0.03	21.8±0.07	17.9±0.06
ECR31	Russia	14.6±0.15	16.1±0.06	15.9±0.07	17.8±0.18	12.8±0.07	21.4±0.03	16.4±0.03	21.6±0.03	20.1±0.03

ECK17	Kyrgyzstan	17.3±0.03	16.2±0.06	16.2±0.12	17.4±0.13	14.2±0.10	14.2±0.07	15.8±0.03	20.9±0.09	18.4±0.07
ECK19	Kyrgyzstan	15.1±0.06	15.8±0.07	12.7±0.07	18.2±0.12	14.0±0.07	22.5±0.03	12.6±0.07	17.9±0.09	16.5±0.15
ECK22	Kyrgyzstan	17.3±0.03	13.2±0.06	16.2±0.12	17.4±0.13	14.2±0.10	14.2±0.07	15.8±0.03	20.9±0.09	18.4±0.07
ECK27	Kyrgyzstan	13.9±0.03	14.2±0.07	15.9±0.06	18.2±0.06	14.2±0.03	22.8±0.03	12.9±0.13	20.1±0.03	20.2±0.06
Reference values**	**Control ATCC 2592**	**14-17**	**14-16**	**14-15**	**16-21**	**12-14**	**22-23**	**13-15**	**18-20**	**16-20**

*– Values indicating antibiotic resistance are highlighted in gray.
** – Values for the control strain ATCC 2592.
***AMO -Amoxicillin;AMP -Ampicillin;MER -Meropenem;PEF -Pefloxacin;STR -Streptomycin;TIC -Ticarcillin+clavalanic acid;FOS -Fosfomycin;CEF -Ceftibuten;CIP -Ciprofloxacin.

IV. Discussion

Fresh vegetables often harbor natural, nonpathogenic epiphytic microorganisms but are exposed to high levels of microbial contamination through contact with soil, dust, and water, as well as handling during harvest or postharvest processing[15]. It is possible that such high numbers of pathogenic and opportunistic species in vegetables are related to violations of cultivation practices and the use of untreated fertilizer from sewage sludge or manure. The presence of clinically significant microorganisms in natural samples may also indicate a high anthropogenic load and pollution of the natural environment.

V. Conclusion

Local screening with prospective testing of fresh vegetables from different traders would be a valuable tool for ecological and monitoring assessments.

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