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**Isolation and molecular characterization of bacteria carrying antibiotic
and disinfectant resistant genes potentially transmitted from irrigation
water to *Capsicum Annuum***

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ABSTRACT

Irrigation water potentially contaminated by the discharge of untreated wastewater into water bodies is a major source of fresh produce contamination with water-borne pathogens including antibiotic-resistant bacteria (ARB) and disinfectant-resistant bacteria (DRB). Contaminated fresh produce can transfer these bacteria and their resistance genes to consumers, particularly when consumed in the raw state. Despite potential health risks, no specific regulations have been established to ensure the quality of irrigation water with respect to ARB and DRB. This study aimed to assess the impact of bacterial contamination on the aquatic ecosystem and its receiving environment caused by the discharge of untreated wastewater in Kosovo. Therefore, ground- and river water used for irrigation, irrigated soil fields, and pepper (*Capsicum Annum*) from five vegetable growing areas were examined for the presence of total- and extended-spectrum beta-lactamase (ESBL)- *Escherichia coli* (*E. coli*) and potential pathogenic and quaternary alkylammonium compounds (QAACs)- tolerant bacteria.

All bacteria were cultured by direct plating and non-selective pre-enrichment cultivation, which increased the detection of numerous genera. *E. coli* were cultured on Tryptone Bile X-Glucuronide (TBX) agar, whereas antibiotic-tolerant ESBL *E. coli* were cultured on TBX agar supplemented with 1 mg L⁻¹ cefotaxime antibiotic (CTX). Potential pathogenic bacteria were cultured on Müller Hinton (MH) agar and quaternary alkylammonium compounds (QAACs)-tolerant bacteria were cultured on MH supplemented with disinfectant BAC-C12 50 µg mL⁻¹ and 100 µg mL⁻¹.

A total of 315 bacteria were isolated and identified from the water (n=262), soil (n=33), and pepper samples (n=20).

Sixty-nine out of 103 isolates (67%) examined from TBX agar were identified as *E. coli* by 16S rRNA gene sequencing and by the presence of β-D-glucuronidase and glutamate decarboxylase genes A and B (*gadAB*), of which 97% from water (n=67) and 3% from soil samples (n=2). No isolates were detected in the pepper samples. We further detected a high prevalence of 50 potential ESBL-producing *E. coli* isolates originating from the water samples (56%; river water, 44%; groundwater). There were no isolates detected in the soil or pepper samples. The non-target bacteria detected in TBX +/- CTX 1 mg L⁻¹ were assigned to the genera *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Bordetella*, *Brucella*, *Citrobacter*, *Enterococcus*,

Klebsiella, *Morganella*, *Proteus*, *Providencia*, *Pseudomonas*, *Siccibacter*, and *Stenotrophomonas*.

A total of 108 disinfectant-tolerant bacteria were examined from MH agar supplemented with the disinfectant BAC-C12, and phylogenetically identified as members of the genera *Aeromonas*, *Morganella*, *Providencia*, and *Pseudomonas*, of which 78% originated from water (n=84), 6% from soil (n=7), and 16% from pepper samples (n=17).

Overall, this study identified several risks that contaminated irrigation water through untreated wastewater discharge constitutes a source of multidrug-resistant bacteria that may enter the food chain through vegetable ingestion.