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SHORT COMMUNICATION

First Isolation of *Exiguobacterium aurantiacum* in Serbia

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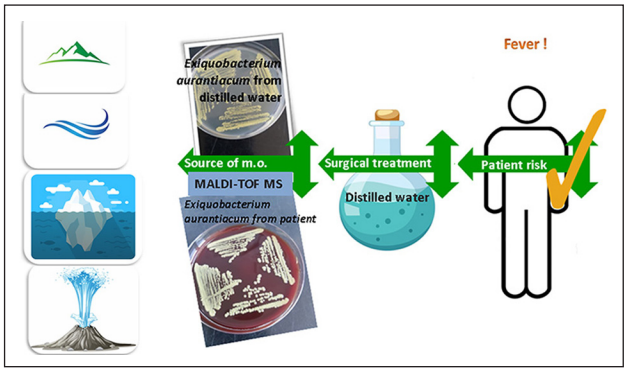
Abstract

Exiguobacterium aurantiacum is isolated from a variety of environmental samples but rarely from patients. The aim of the study was to represent isolation of unusual bacterial strains that could cause infection in patients. Final identification was performed using matrix-assisted description/ionization time-of-flight mass spectrometry (MALDI-TOF). Two isolates strains of *E. aurantiacum* were isolated, one isolate from distilled water used during surgical treatment and the second one from a patient with bacteremia after radical prostatectomy, both sensitive to all tested antimicrobials. Environmental strains could cause infection, especially in immunocompromised patients; therefore, rare bacteria testing is required, in which identification special assistance is provided by an automated system MALDI-TOF.



K e y w o r d s: *Exiguobacterium aurantiacum*, identification, MALDI-TOF

Exiguobacterium aurantiacum belongs to the genus *Exiguobacterium,* initially isolated from potato-processing effluent in 1983 by Collins et al. (1983), as the first species of this genus. Later, members of this genus have been isolated from various environments, including glaciers in Greenland and Siberia and hot springs in Yellowstone, today, counting 17 species (Vishnivetskaya et al. 2009; Ramesh and Pandey 2017; Strahsburger et al. 2018).

E. aurantiacum are aerobic, motile, non-spore-forming Gram-positive short coryneform bacilli, catalase- and DNase-positive, oxidase-negative, and alkaliphilic. These bacilli grow on nutrient agar at pH 10 and in the presence of NaCl 6% w/v (halotolerant), reduce nitrate to nitrite, and metabolize glucose fermentatively. Biochemically very active, they produce acid from glucose, galactose, glycerol, maltose, mannitol, and sucrose but not from L-arabinose, dulcitol, lactose, melezitose, raffinose, rhamnose, sorbitol, or xylose. Sometimes these bacteria produce acid from fructose and salicin, and hydrolyse starch, casein and gelatin. They form orange-yellow pigmented colonies on blood agar (Takemura et al. 2009). *E. aurantiacum* has been very rarely reported to cause infections in humans worldwide, such as bacteremia in the UK, corneal ulcers in India, and pneumonia in China (Pitt et al. 2007; Chen et al. 2017; Jain and Kamble 2018), however, in most works, the origin of the infection is still unclear.

So far, *E. aurantiacum*, was unidentified from human infections in Serbia. Here, we report the first isolation of *E. aurantiacum* in Serbia, still causing bacteremia in a patient after surgery.

In December 2020, two samples, distilled water and patients' blood sample, from the Department of Urology, Clinical Center of Vojvodina, Novi Sad, Serbia,

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were submitted to the Department of Sanitary Bacteriology of the Institute of Public Health of Vojvodina, Novi Sad, Serbia, for microbiological analysis.

First, distilled water was brought on routine microbiological examination according to European Pharmacopoeia 9th edition. 100 ml of distilled water was aseptically filtrated through the membrane filter system using sterile mixed cellulose esters membrane filters 0.45 μm pore size (Sartorius, Goettingen, Germany) after which membrane filter paper was placed on R2A agar (Oxoid, Basingstoke, Hampshire, UK), and incubated for seven days in 22°C. The colonies were then sub-cultured on blood agar (Oxoid, Basingstoke, Hampshire, UK), and further examination and confirmation were performed using matrix-assisted description/ionization time-of-flight mass spectrometry spectrometry (MALDI-TOF-MS) (Brucker, Carteret, NJ, USA).

The following day, a blood sample was taken from an adult male, aged 65 years, who became febrile (38.4°C) after a radical prostatectomy. The samples were incubated in aerobic and anaerobic blood culture bottles (BioMerieux, Marcy l'Etoile, France). Gram-positive bacilli have been found in both bottles and were subsequently identified as *E. aurantiacum* using MALDI-TOF-MS. The aerobic and anaerobic blood culture bottles were inoculated on nutrient agar (HiMedia, India), incubated for 24 h at 35 ± 1°C. The colonies were transferred to a highly polished stainless steel plate to be analyzed in vacuum. In this particular application of the MALDI-TOF-MS method, the surface-associated molecules of the cell yield a species-specific mass spectral profile that can be aligned with profiles in an existing database (Keys et al. 2004). Both isolates matched the *E. aurantiacum* profile, with characteristic mass ions at 772 and 1,379 Da reinforcing this identification.

Antimicrobial susceptibility testing was performed according to EUCAST Broth Microdilution in accordance to ISO 20776-1, where the following antimicrobial drugs were tested: penicillin, meropenem, gentamicin, ciprofloxacin, rifampicin, vancomycin, tetracycline, erythromycin, clindamycin according to EUCAST 2020, Clinical Breakpoint v.10.0 for nonspecies related MIC breakpoint.

Two isolates of *E. aurantiacum* were obtained, the first one from distilled water used during surgery in the Department of Urology, Clinical Center of Vojvodina, Novi Sad, Serbia, and the second from the patient with bacteremia after radical prostatectomy.

Antimicrobial susceptibility testing was performed by using the microdilution method, and the MIC results were as follows: penicillin (≤0.064), meropenem (≤0.064), gentamicin (≤ 0.125), ciprofloxacin (≤0.125), rifampicin (≤0.064), vancomycin (≤0.125), tetracycline (≤0.125), erythromycin (≤0.125), clindamycin (≤0.125).

During the identification of bacteria empirical therapy, ceftazidime, according to the protocol for fever, was applied. As bacterial strain was susceptible to all tested antimicrobial drugs, the patient responded well to administrated therapy, and microorganism was not recovered from subsequent blood culture specimens. Nevertheless, the mentioned bacterium was again isolated from a repeated sample of distilled water, indicating inadequate preparation of such water, which became bacterial contamination originating from the environment.

E. aurantiacum, a microorganism widely distributed in nature, is recognized as a rare human pathogen affecting mostly sensitive population groups, especially with an impaired immune system. It is difficult to be identified based on the traditional biochemical method due to a substantial similarity to *Cellulomonas*/*Microbacterium* spp. (Pitt et al. 2007). Also, it can be misidentified as *Oerskovia xanthineolytica* when API Coryne kit (BioMérieux, Marcy l'Étoile, France) is used (Kenny et al. 2006). Inadequate and partial microbial diagnosis can lead to an inaccurate conclusion. Therefore, it is necessary to include modern automated systems, such as MALDI-TOF, which helps in everyday work to identify bacteria to the species level. Identifying environmental isolates to the species level is crucial for obtaining a correct coverage of the health impact caused by these microorganisms.

Based on literature reviews, *E. aurantiacum* is isolated occasionally from various human clinical specimens like blood, broncho-alveolar lavage fluid, or corneal ulcers in only a few countries worldwide, such as the UK, China, and India. In most cases, the origin of the infection remained unclear or unknown, while in pneumonia, the inhalation route was confirmed, and in corneal ulcer, the connection with contact lenses was certified (Pitt et al. 2007; Chen et al. 2017; Jain and Kamble 2018). Our research concluded that the source of bacteria was distilled water, especially since the same pathogen was reisolated from the repeated samples of distilled water. Since the ability of biofilm production has been proven for *Exiguobacterium* sp. in several studies (Chen et al. 2017; Gutiérrez-Preciado et al. 2017), we believe that this is the most probable reason why this bacterium was reisolated. As a corrective measure, the remediation of the water distillation apparatus was proposed. After this action, the distilled water samples were correct until micro contamination with potential pathogens capable of creating a biofilm occurs again, and there are more and more of them in the environment. This study is one of the rare examples, which try to elucidate the link between the environment and the clinic.

Pitt et al. (2007) determined susceptibility of *E. aurantiacum* to all tested antimicrobial drugs, as we did in our study, while Jain with associates (2018) and Chen

with collaborators (2017) determined the susceptibility of the bacteria to penicillin, meropenem, gentamicin, ciprofloxacin and rifampicin, and their resistance to tetracycline, erythromycin, clindamycin. The susceptibility to vancomycin was railed in these two studies; the bacteria were sensitive according to Chen et al. (2017) and resistant in the publication of Jain and Kamble (2018). It has been shown that environmental bacteria are involved in the horizontal flow of resistance genes. Especially soil microorganisms contribute to or acquire resistance determinants from pathogens (Yang et al. 2014).

Authors from the United Kingdom have reported similar findings of *E. aurantiacum* isolated from blood culture (Pitt et al. 2007), but this is the first report of such very rare isolate in Serbia.

Even if *E. aurantiacum* is unusual cause of human infection, this report describes the ability of environmental strains to cause infection, especially in immunocompromised patients, and emphasizes the need for testing rare bacteria, in which identification special assistance is provided by an automated system such as MALDI-TOF.

The isolation of *E. aurantiacum* from a sample of distilled water arouses a big doubt about the hygiene of the used device, which can cause a severe infection in patients who are subjected to the treatment. As a potential pathogen, *E. aurantiacum* should attract more attention.

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Contribution

Each author contributed equally to the development of the manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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