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**RHIZOBIUM RADIOBACTER – A NEW PATHOGEN OF
HEALTHCARE-ASSOCIATED INFECTIONS**

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Abstract. *This paper presents the results of a study of cases of catheter-related bloodstream infections in patients with a central venous access catheter installed for long-term infusion of medications to treat the main disease. It is known that intravascular devices are a risk factor for the development of catheter-related bloodstream infections (CRBSI). Therefore, conducting an epidemiological study of implanted vascular catheters is an important component of infection control in healthcare facilities. During the bacteriological study of blood samples from the central catheter (using BACT/ALERT FA Plus, the BACT/ALERT 3D blood culture system and nutrient media), 19 cultures were obtained, which were identified as Rhizobium radiobacter using the automated VITEK-2 Systems (bioMerieux, Inc). In the vast majority, all isolated strains demonstrated high sensitivity to antibiotics. However, 100% resistance was found to some antibiotics. Isolation of Rhizobium radiobacter from the blood of patients encourages the expansion of the etiological structure of catheter-associated bloodstream infections and confirms the importance of epidemiological surveillance of implanted intravascular devices.*

Keywords: healthcare-associated infections, Rhizobium radiobacter infection control, antibiotics, antibiotic resistance.

Introduction. The problem of prevention and treatment of healthcare-associated infections (HAI) does not lose its relevance. According to the World Health Organization, every tenth patient is harmed while receiving medical care in a hospital setting (Roychoudhury, 2024).

A significant place among all HAI belongs to catheter-related bloodstream infections (CRBSI). This is due to the widespread use of vascular catheters for therapeutic and diagnostic procedures. However, the use of other intravascular devices can also lead to the development of bloodstream infections. According to the European Center for Disease Prevention and Control, in 2017, 44% of BSI episodes were catheter-related in the structure of the incidence of HAI in intensive care unit patients in the European Region (European Centre for Disease Prevention and Control, 2019). The results of a pilot study on the simultaneous prevalence of healthcare-associated infectious diseases, which was conducted in five regions of Ukraine in 2021, showed that 421,800 patients were infected in the hospital, the number of registered cases of bloodstream infections was 2.3% of the total number of HAI (Public health center of MoH of Ukraine, 2021). The low percentage of registered cases of CRBSI is explained by the lack of organized surveillance for the control and registration of cases of bloodstream infections, insufficient amount of performed blood culture tests, limited capabilities of medical institutions to diagnose these infectious complications, and in some cases - deliberate avoidance of documenting the presence of a case of this type of HAI.

Currently, the leaders among pathogens that cause the development of HAI are gram-negative bacteria. The World Health Organization (WHO) updated the list of priority pathogens in 2024, which included 24 pathogens representing 15 families of the prokaryotic kingdom. Among them, most microorganisms are multi-resistant to many antibacterial drugs, including reserve group antibiotics. Infections caused by such microorganisms lead to high mortality.

According to WHO, in 2019, 4.95 million people died from infections caused by gram-negative multi-resistant pathogens (WHO, 2024).

In the etiological structure of CRBSI, the most prevalent agents are gram-negative non-fermenting microorganisms. The natural habitat of the most significant bacteria in disease development from the genera *Pseudomonas* and *Acinetobacter* consists of soil and water rich in organic residues from open water bodies. In the hospital environment, these microorganisms often colonize areas that are constantly moistened (sanitary equipment, tiled floors and walls, etc.). The extraordinary plasticity of these microorganisms' genetic apparatus makes them resistant to various adverse factors in the surrounding environment and to disinfectants.

However, recent reports indicate new microorganisms responsible for the development of hospital infectious complications, especially in immunocompromised patients. Are they equally dangerous? Will they increase the list of priority pathogens in the future?

The aim of study. Based on studies examining the etiology of a series of CRBSI cases, this research aims to assess the potential for adding to the list of HAI pathogens a new representative of gram-negative bacterial microflora, *Rhizobium radiobacter*.

Materials and methods. During March 2024 - December 2024, 30 cases of prolonged subfebrile temperature were observed in patients with signs of secondary immunodeficiency who were undergoing long-term outpatient treatment in a municipal medical institution. All patients were fitted with a CVC for the long-term administration of medical drugs for their primary condition. Given that intravascular devices are a risk factor for bloodstream infections, blood samples were collected from the CVC for bacteriological examination and sent to the bacteriological laboratory. The transportation of the samples was conducted in accordance with Order №354 from the Ministry of Health of Ukraine dated 02/21/2023, On the organization of prevention of nosocomial infections (Law of Ukraine, 2023).

Blood samples were cultivated in commercial vials for growing aerobic and anaerobic BACT/ALERT FA Plus and incubated in a BACT/ALERT 3D blood culture system. The growth of microorganisms in the vials was present in almost all cases for 3 days. In most cases, the aerobic vial became positive even earlier. The material from positive vials was then cultivated on BAP, CBA, Endo medium, MSA, Enterococcus Selective Agar, CHROMagar Orientation. Quality control of nutrient media was carried out in accordance with the recommendations of manufacturers, which are set out in the product certificates. In cases of positive test results, the morphological and cultural characteristics of microorganisms were studied. Species identification and antimicrobial susceptibility testing of isolated cultures was performed using the automated VITEK-2 Systems (bioMerieux, Inc).

During the study of the biochemical properties, fermentation was taken into account for 45 biochemical substrates, namely: Ala-Pne-Pro-arylamidase (APPA), adonyl (ADO), L-pyrrolidone arylamidase (PyrA), L-arabit (IARL), D-cellobiose (dCEL), beta-galactosidase (BGAL), beta-N-acetylglucosaminidase (BNAG), glutamylarylamidase (AGLTp), glucose (dGLU), gamma-glutamyltransferase (GGT), maltose (dMAL), mannitol (dMAN), mannose (dMNE), beta-glucosidase (BGLU), beta-xylosidase (BXYL), beta-alanine arylaminadase (BALap), L-proline arylamidase (ProA), as well as lipase (LIP), sucrose (SAC), palatinose (PLE), tyrosine arylamidase (TyrA), sorbitol (dSOR), trehalose (dTRE), citrate (CIT), malonate (MNT), lactate (ILATk), keto-D-gluconate (5KG), succinate (SUCT), phosphatase (PHOS), acetylglactosaminidase (NAGA), alphagalactosidase (AGAL), ornithine decarboxylase (ODC), glucose fermentation ability (OFF) and hydrogen sulfide (H₂S) production.

The assessment of the antibiotic susceptibility of the studied bacterial cultures was carried out in accordance with the EUCAST recommendation for ECOFF for *Acinetobacter* (EUCAST, 2025). The determination of susceptibility was carried out to the following antibiotics: imipenem, meropenem, doripenem, amikacin, gentamicin, tobramycin, levofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole.

Results. Peripheral vascular catheterization is one of the most common invasive procedures in medical practice, necessary for a number of therapeutic and diagnostic procedures. However, the use of intravascular catheters is a risk factor for the development of bloodstream infections through direct entry of microorganisms into the bloodstream. Among 30

examined blood samples from CVCs, 19 positive results were obtained, which is 63.3%. Microscopic examination of smears prepared from pure cultures of microorganisms grown on nutrient media demonstrated the presence of gram-negative non-spore-forming rods, which were located mainly singly or with a tendency to dense clusters (fig. 1).

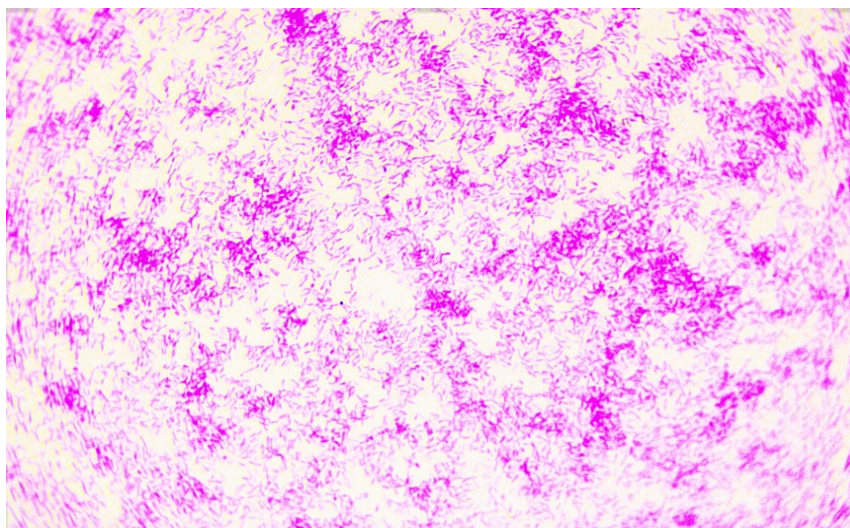


Fig. 1. *Rhizobium radiobacter* microscopy (magnification x1000)

The next day after incubation on BAP and CBA, growth of mucous colonies with a diameter of 1 mm was observed, as well as poor growth on Endo medium. After 48 hours of incubation in a thermostat at a temperature of 37°C, growth on Endo medium increased, but the colonies were small with a metallic sheen. The results of the oxidase and urease tests were positive.

Based on the obtained biochemical properties, all isolates were identified as *Rhizobium radiobacter* with a probability of 99% by the automated system VITEK-2 Systems (bioMérieux, Inc) using cards for the identification of gram-negative microorganisms (GN).

Analysis of the biochemical properties of the isolated cultures showed that the vast majority of strains had biochemical properties typical of the species. However, several strains differed in terms of fermentation of certain substrates (Table 2).

Table 2

Differentiation of *Rhizobium radiobacter* strains by biochemical properties

Substrate	Fermentation		No fermentation	
	№ of strains	% of strains	№ of strains	% of strains
proA	10	52,6%	9	47,4%
ADO	11	57,8%	8	42,2%
dTRE	3	15,7%	16	84,3%
BGAL	5	26,3%	14	85,7%
SAC	5	26,3%	14	85,7%
GLyA	6	31,6%	13	68,4%
PLE	4	21%	15	79%
BXYL	11	57,8%	8	42,2%

The results of determining the sensitivity of the isolated strains to antibiotics are illustrated in Figure 2.

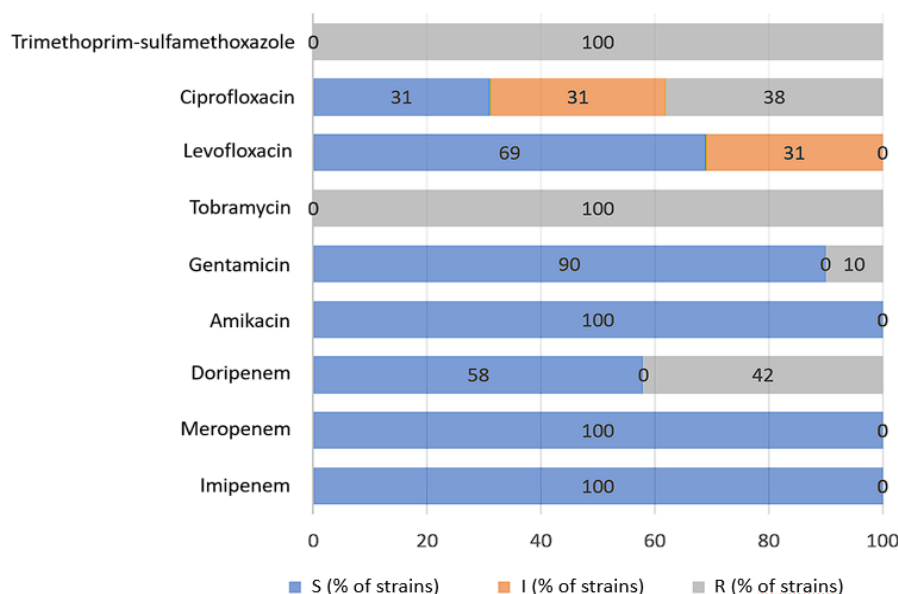


Fig. 2. Sensitivity of isolated strains of *Rhizobium radiobacter* to antibiotics

As shown in Fig. 2, most of the isolated strains of rhizobium were sensitive to carbapenems. All isolated isolates were sensitive to imipenem and meropenem. The sensitivity to doripenem (carbapenem of the 3rd generation) was 58%. It is also important that these antibiotics belong to the watch group (B) and can be prescribed only after proven ineffectiveness of antibiotics from the access group. The sensitivity of the isolated strains of *R. radiobacter* to aminoglycoside antibiotics is of considerable interest. The sensitivity to the aminoglycoside of the 2nd generation – to the gentamicin - was at the level of 90%, to amikacin all tested isolates demonstrated full sensitivity. At the same time, 100% of the isolated strains were resistant to tobramycin.

As for fluoroquinolone drugs, the sensitivity of *Rhizobium radiobacter* strains was 31% to ciprofloxacin and 69% to levofloxacin. The relatively high percentage of resistant strains to levofloxacin is noteworthy, since since 2023 this drug has been classified as a reserve drug and is prescribed only based on vital signs.

The above-mentioned sensitivity of the isolated *Rhizobium radiobacter* isolates was obtained using the automated VITEK-2 system using a card with an AST 331 reagent kit, which is used to determine antibiotic sensitivity for the group of non-fermenting gram-negative microorganisms. It is clear that the data obtained are insufficient to generalize the results regarding the resistance of rhizobia to antibiotics. Therefore, we continued to study the sensitivity to antimicrobial drugs by the disk diffusion method. The results of the study demonstrated high sensitivity of isolated strains of bacteria of the genus *Rhizobium* to inhibitor-protected and –unprotected aminopenicillins. Thus, about 96% of isolated strains were sensitive to amoxicillin and amoxicillin/clavulanate. 100% of rhizobium strains were sensitive to the third-generation cephalosporins ceftriaxone and cefoperazone/sulbactam. However, only 56% of strains were susceptible to cefoxitin. Cefepime, ceftazidime and ceftazidime/avibactam were ineffective against 95% of *R. radiobacter* strains.

Discussion. The implementation of Order No. 1614, On the Organization of Infection Prevention and Infection Control in Healthcare Institutions and Institutions Providing Social Services/Social Protection of the Population, marked a critical step in Ukraine's efforts to improve HAI registration and control. Central to this initiative is the standardized documentation of invasive procedures, including peripheral and central vascular catheterizations. Routine epidemiological surveillance of HAIs remains essential for timely detection, microbial identification, and informed antimicrobial stewardship.

Among patients receiving intravenous therapy, a subset requires long-term cyclic or course-based drug administration. For these individuals, central venous catheters (CVCs) are utilized. These devices, designed to integrate into patient tissue, are believed to reduce infection risk by creating a barrier against skin-colonizing pathogens. However, extended

catheter use can predispose patients to intraluminal infections, often stemming from breaches in aseptic technique during catheter maintenance. Less commonly, hematogenous catheter contamination may occur, originating from distant infection foci such as pneumonia, urinary tract infections, or fungemia (Small, 2015; The Joint Commission, 2013; Balasoiu, A., 2022).

Despite state-level surveillance efforts, CRBSI control remains suboptimal. To some extent, this is due to the fact that patients with installed intravascular devices for long-term drug administration spend most of their time outside the hospital and there is no certainty about proper care of the CVC site. Accordingly, early detection of symptoms of local inflammation (redness, swelling, etc.) is difficult.

R. radiobacter (formerly *Agrobacterium tumefaciens*) is a gram-negative, non-spore-forming, motile aerobic rod, usually a phytopathogen, causing the development of plant tumors. Its natural habitat is soil, along with other non-fermenting bacteria. Human disease caused by representatives of the genus *Rhizobium* is an infrequent phenomenon. There are five known species of bacteria of the genus *Rhizobium*, namely: *Rhizobium radiobacter*, *Rhizobium rhizogenes*, *Rhizobium rubi*, *Rhizobium undicola*, *Rhizobium vitis*. Interestingly, *R. radiobacter*, with its low virulence, is the only species involved in the development of diseases in humans (Aujoulat, 2015).

The earliest report of *R. radiobacter* causing endocarditis following aortic valve replacement dates back to 1980 (Plotkin, 1980). Since the early 2000s, increasing numbers of infections have been reported, including endocarditis post-heart valve replacement, bacteremia in cancer patients, neonatal sepsis, and, more rarely, UTIs in dialysis patients, nosocomial pneumonia, and brain abscesses (Tiwari, 2015; Fatma, 2024; Sood, 2010). In addition, episodes of the participation of these microorganisms in the development of urinary tract infections (in patients requiring hemodialysis), nosocomial pneumonia, brain abscesses, etc. have been confirmed. The placement and presence of plastic devices in the human body is a major factor in the development of infections caused by this pathogen. This is explained by the high adhesion properties of rhizobia to plastic/silicone surfaces due to the production of extracellular mucus (Lai, 2004).

The detection of *R. radiobacter* in blood samples of 19 patients led to the assumption of the presence of an intrahospital reservoir of infection and prompted an epidemiological investigation. However, a thorough bacteriological examination of the hospital environment where intravascular devices were installed did not yield positive results. According to the literature, one of the possible sources of rhizobia is the gastrointestinal tract, from where they can subsequently spread into the bloodstream. In addition, the outpatient nature of the treatment of the examined patients suggests the presence of many sources of infection during the patients' stay outside medical institutions (Wang, 2019).

Whether these cases constitute a hospital outbreak remains inconclusive. Nonetheless, the frequent detection of *R. radiobacter* in CVC blood cultures underscores its potential role in expanding the known etiological spectrum of CRBSI. *Rhizobium* spp. should be regarded as opportunistic pathogens capable of causing bloodstream infections in immunocompromised individuals—those with cancer, organ transplants, HIV, or undergoing corticosteroid or cytostatic therapy.

The general susceptibility of *R. radiobacter* to antibiotics suggests these infections are currently manageable. However, the notable aminoglycoside resistance patterns—especially to tobramycin—warrant further investigation, not only for treatment purposes but also for use as diagnostic markers.

Currently, the list of antibiotics for susceptibility testing of *R. radiobacter* is undefined. EUCAST guidelines, while authoritative, do not include this organism in their 2025 update, creating ambiguity around appropriate testing standards. This gap requires further discussion within the microbiological and infectious disease community.

Conclusion. The results of the study confirm the need and importance of epidemiological surveillance of the use of intravascular catheters and other devices that are incorporated into vessels or other cavities of the human body for a long time for therapeutic and diagnostic purposes. At the same time, the possibility of isolating emergent species of

bacteria should be considered, especially in patients with primary or secondary immunodeficiencies, and comprehensively study each positive bacteriological culture. The repeated isolation of *R. radiobacter* strains from the blood of CVCs emphasizes the need for further research into its virulence factors, adhesive capabilities, and biofilm-forming properties. Advanced diagnostic systems—including automated blood culture systems and microbial analyzers—should be employed to ensure precise identification and resistance profiling in each CRBSI case.

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RHIZOBIUM RADIOBACTER – НОВИЙ ЗБУДНИК ІНФЕКЦІЙ, ПОВ'ЯЗАНИХ З НАДАННЯМ МЕДИЧНОЇ ДОПОМОГИ

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Анотація. У статті представлені результати проведеного дослідження випадків катетер-асоційованих інфекцій кровотоку у пацієнтів з встановленим центральним портом для проведення довготривалої інфузії медичних препаратів з метою лікування основного захворювання. Відомо, що встановлені внутрішньо-судинні девайси є фактором ризику розвитку КАІК, тому проведення епідеміологічного дослідження встановлених судинних катетерів є важливим компонентом інфекційного контролю закладів охорони здоров'я. При проведенні бактеріологічного дослідження крові з центрального порту (із використанням BACT/ALERT FA Plus та гемокультиватора BACT/ALERT 3D та поживних середовищ) було отримано 19 позитивних культур, які, за допомогою автоматизованої системи VITEK-2 Systems (bioMérieux, Inc) були ідентифіковані як *Rhizobium radiobacter*. В переважній більшості всі виділені штами продемонстрували високу чутливість до антибіотиків, проте, до деяких антибіотиків виявили 100% резистентність.

Виділення *Rhizobium radiobacter* із крові пацієнтів спонукає до розширення етіологічної структури катетер-асоційованих інфекцій кровотоку та підтверджує важливість епідемічного нагляду за встановленими внутрішньосудинними девайсами.

Ключові слова: інфекції, пов'язані з наданням медичної допомоги, *Rhizobium radiobacter*, інфекційний контроль, антибіотики, антибіотикорезистентність.

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