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SIGNIFICANCE OF ACHROMOBACTER XYLOSOXIDANS IN ACUTE INTESTINAL INFECTIONS

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ABSTRACT

In this article, we aim to provide information about a unique case that was first discovered in Uzbekistan from isolates of a patient with acute intestinal infection. A rarely identified microorganism, Achromobacter xylosoxydans, was detected using molecular genetic methods. Consequently, a literature review of this microorganism was conducted. It has been determined that Achromobacter xylosoxydans, as well as other Achromobacter species, can cause a wide range of infections in hosts with underlying diseases, as well as independent diseases of various organs and systems with severe complications. Their infrequent occurrence and continuously evolving taxonomy make it challenging to determine their clinical features, risk factors for infection, and adverse outcomes. Due to the antimicrobial resistance of Achromobacter species, they are difficult to treat.

Key words: Achromobacter, Achromobacter xylosoxydans, antibiotic resistance, feces, bacteremia, MALDI-TOF, diagnosis, infection.

INTRODUCTION

Relevance. In 1923, for the first time, the Committee of the Society of American Bacteriologists (now known as the American Society for Microbiology)

described *Achromobacter* microorganisms as "Non-pigment-forming, motile or non-motile gram-negative bacteria found in water (various water sources) and soil" [1]. The close resemblance of the genus *Achromobacter* to the genus *Alcaligenes*, both of which belong to the family *Alcaligenaceae* of the order *Burkholderiales*, led to the attribution of several *Achromobacter* species to the genus *Alcaligenes* and vice versa. According to the experts of Bergey's International Society for Microbial Systematics (BISMiS) in 2015, the genus *Achromobacter* includes only 3 species: *Achromobacter xylosoxidans* (the type species, which includes 2 subspecies - *Achromobacter xylosoxidans subsp. xylosoxidans* and *Achromobacter xylosoxidans subsp. denitrificans*), *Achromobacter piechaudii*, and *Achromobacter ruhlandii* [12]. The genus *Achromobacter* currently includes 19 officially designated species, most of which have been described over the past decade.[2] To date, fifteen species have been identified from clinical samples, including *Achromobacter xylosoxydans, Achromobacter denitrificans, Achromobacter ruhlandii, Achromobacter piechaudii, Achromobacter animicus, Achromobacter mucicolens, Achromobacter pulmonis, Achromobacter insolitus, Achromobacter spanius, Achromobacter deleyi, Achromobacter aegreficans, Achromobacter insuavis, Achromobacter anxifer, Achromobacter dolens u Achromobacter marplatensis*. *A. xylosoxydans* is the most common species in the world, isolated from clinical samples, including those obtained from individuals with cystic fibrosis (CF). The distribution of other species shows geographical diversity. A. *ruhlandii* is the second most common species in the Americas [9-11], while *A. dolens* and *A. insuavis* are more common in Europe [12-14]. The clinical significance of the species variability is not well characterized by *Achromobacter xylosoxydan*s - a gram-negative rod.

The pathogen can cause diseases such as meningitis, sepsis, or inflammation of the heart's inner membrane (endocarditis) in people with weakened immune systems. In addition, there may be bacteriemia, i.e. the appearance of many bacteria in the blood. *Achromobacter xylosoxydans* infection also plays a role in cystic fibrosis, a previously incurable metabolic disease. Mucoviscidiosis can later lead to other secondary diseases, such as diabetes and osteoporosis. The pathogen was first isolated in patients with middle ear inflammation.

Purpose. To study the features of *Achromobacter xylosoxydans* and its significance in acute intestinal infections as a result of testing the genotype of the isolate isolated from the feces of a patient suspected of acute intestinal infection in Tashkent by the method of 16S rRNA sequencing.

Materials and methods. In 32 patients admitted to the Tashkent city infectious diseases hospital with the diagnosis of acute intestinal infection, feces

were bacteriologically examined and 32 shigella isolates were isolated. Isolates were delivered to the Center for Biomedical Technologies of the Tashkent Medical Academy for final diagnosis. **Molecular-genetic identification of bacteria was carried out in the following stages: DNA isolation.** Bacterial DNA was isolated from 31 pure isolate cultures (Shigella spp.) by salt extraction. The quality and quantity of the obtained DNA were tested using the Implen NanoPhotometer N (Implen, Germany) spectrophotometer according to the manufacturer's instructions. All samples of DNA solutions were stored at -20°C. **Amplification of the 16S rRNA gene by PCR.** 16S gene amplification was performed on a C1000 amplifier (BIO-RAD, USA) using a set of RV PCR reagents (Sintol, Russia) and a pair of primers in the following sequence: F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-AAGGAGGTGATCCAGCCGCA-3'). The PZR reaction program was as follows: initial denaturation at 94°C - 30 minutes, 55-600°C - 30 seconds for 35 cycles, 57°C - 20 seconds, 72°C - 40 seconds, and final denaturation at 72°C - 1 minute. The obtained amplifiers were detected by electrophoresis detection in 2% agarose gel. The PCR purification of the product from the agarose gel was carried out using the Cleanup St Gel reagent kit for purifying DNA from the agarose gel (Eurogen, Russia), according to the manufacturer's instructions. The sequencing reaction was carried out on a purified PCR product using the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher). The sequencing product was cleaned using the BigDye XTerminator cleaning kit and placed in the sequener. Sequencing reactions and cleaning methods of the sequencing products were carried out according to the set instructions. The obtained data on the sequencing of the 16S gene of the shigella DNA was processed using the Chromas software. After cleaning the chromatogram sequence from poorly informative parts, the DNA nucleotide sequence was compared to the BLAST nucleotide NCBI database.

Results and discussion

Of the 32 patients, 32 *Shigella* isolates were identified using a bacteriological method. When amplifying the 16S rRNA gene using PCR, 31 isolates were compared to *Shigella (Shigella boydii, Shigella flexneri, Shigella zonne*). Isolate number 6 (105) proved to be another opportunistic microorganism - *Achromobacter xylosoxidans* with 97.69% confirmation. When staining according to Gramm, the colors were gram-negative, medium-sized sticks.

Literature has shown that this microorganism causes many diseases, including intestinal infections. Here are a few examples from the literature that show that this bacterium has such a severe (infectious, epidemiological) infectious effect. In the

environment, the microbe is usually found in the soil or in various water sources. Spreading in various water sources may be the cause of the infection of this patent with this microbe. But that's only a supposition, and the probability is great. Epidemiological data indicate that water and moist soil are natural sources of *A. xylosoxidans* infection [2, 4]. For nascomal infections, Ahromobacter species were restored from ventilators, humidifiers, "sterile" salt water, [7] disinfectant solutions,[13] vascular fluid, and irrigation and dialysis solutions [2, 3]. Environmental sources such as well water, musk water, swimming pools, and infant formulas were also identified [2,3,20].

From the literature, it was found that the microbiota is one of the film-forming agents in the lungs of sick people. The film prevents the correct penetration of salts and water into the lung cells. This leads to a disruption in the water-salt balance of the cell [25,29]. This mechanism of pathogenesis may also play a role in the gastrointestinal tract, causing symptoms of acute intestinal diarrhea.

Yorn-Xendrik Vaytkamp (2000) gives the following characteristics: Achromobacter xylosoxydans - aerobic, mobile, oxidazo-and catalase-positive, non-fermenting lactose, gram-negative rod. This organism was briefly classified as *Alcaligenes*, but was recently reclassified as *Achromobacter*.[1] *A. xylosoxydans* was isolated from blood, spinal fluid, feces, urine, sputum, abdominal fluid, skin, discharge from ears, wounds, abscesses, bones, joints, endocardium, and central venous catheters [23,24,25]. Most published clinical reports on A. xylosoxydans describe nosocomial infections in patients with weakened immune status [2, 3, 5-19]. Mortality ranges from 3% for primary or catheter-associated bacteriemia to 80% for neonatal infection [6].

Achromobacter xylosoxidans was thoroughly studied by foreign scientists such as Y Igra-Siegman et.al and others [26,27,32]. They studied the phenotypic and genotypic properties of the bacteria and described the identified A. xylosoxydans from bacterial isolates. For example, blood samples were inoculated in Bactec Peds Plus/F flacons (soybean casein soup enriched with CO2) and treated using a non-radiometric blood cultivation system (Bactec 9240; Becton Dickinson Microbiology Systems, Sparks, Maryland). The isolates were identified using the RapID NF Plus system (REMEL, Norcross, Georgia), which provided biotype numbers identifying the isolates as A. xylosoxydans. Classical cultivation methods and biochemical tests confirmed this identification: the microorganism formed pigment-free colonies on the McConkey agar and was oxidase-positive and indole-negative in point biochemical tests. The isolates were frozen at -70°C before transplantation to test for sensitivity and gel electrophoresis in the pulsed field (PFGE). Micronutrient sensitivity tests. Antimicrobial MPCs were

determined in a cation-corrected Muller-Hinton broth (BBL; Becton Dickinson Microbiology Systems) in Sensititre (Trek Diagnostic Systems, Westlake, Ohio) microenrichment tablets using an inoculum density of 5 CFU/m each at a size of 3.8×10 inches. The tablets were incubated in the air at a temperature of 35°C for 24 hours. Sensitivity reports were conducted in accordance with the requirements of the National Committee for Clinical and Laboratory Standards [28].

Genomic DNA analysis using the PFGE method. Genomic DNA was extracted from log phase cultures of *A. xylosoxydans* isolates grown in "brain-heart" infusion broth (BBL; Becton Dickinson Microbiology Systems), prepared on low-melting agaric pills and separated by the Xba I enzyme (New England Biolabs, Beverly, Massachusetts) for 24 hours [29]. A standard scale of lambda bacteriophage DNA sizes (Bio-Rad Laboratories, Hercules, California) was used. Electroforesis was performed using the GenePath (Bio-Rad) system. The gels were stained with ethidium bromide and photographed in UV light using a computerized documentation system Gel Doc 2000 (Bio-Rad). According to the criteria, isolates were considered clonal-related if there were less than 3 differences in fragments. The PFGE of genomic DNA fragmented by the Xba I enzyme is a recognized method for reproducing the epidemiological typing of *A. xylosoxydans* [31]. *Achromobacter* field strains exhibit a wide range of restriction fragment length polymorphisms [18,23,30].

Let's give an example of a historical clinical event associated with Achromobacter xylosoxydans infection. This information will help you get an idea of the diseases and complications that this conditionally pathogenic microbium can cause. *Achromobacter xylosoxydans* (formerly *Alcaligenes xylosoxydans*) is a rare but important cause of bacteriemia in patients with weakened immune status, and strains are usually resistant to antimicrobial therapy. In a patient with immunodeficiency and hyperimmune globulin M syndrome, 14 episodes of A. xylosoxydans bacteriemia were registered. A 1-month-old child developed chronic otitis media, frequent upper respiratory tract infections. By the age of 9 months, he developed meningitis with negative seeding outcome, bilateral pneumonia, and persistent neutropenia. There was no immunodeficiency in the family history. The child was prescribed intravenous immunoglobulin and glucocorticoid therapy for significant lymphoproliferation. Other medical problems include entropathy with protein loss, mallabsorption of parenteral nutrition, recurrent bilateral otitis media, recurrent pneumonia, hypersplenism, and recurrent pancreatic lymphadenitis. At the age of 12, *A. xylosoxydans* was first isolated from the patient's blood obtained through the central venous catheter. He subsequently had another 13 documented cases of A. xylosoxydans bacteriemia. Each of these episodes was accompanied by

multiple symptoms, including subfebrile, headache, nausea, diarrhea, hematopoiesis, abdominal pain, and dizziness. The patient exhibited a general enlargement of lymph nodes due to IM-related lymphoid hyperplasia, as well as further enlargement and inflammation of specific lymph nodes, often during bacteriemic episodes. Antibiotic sensitivity testing and gel electrophoresis in the pulse field showed that *A. xylosoxydans* single strain caused recurrent bacteriemia in this patient. Each episode of A. xylosoxydans infection was treated with imipenema and tobramicin intravenously or individually. Each course of therapy lasted 7-14 days and led to rapid clinical improvement in blood infection and sterilization symptoms [32].

Ahromobacter infections occur not only in patients with immunodeficiency, as previously thought. The risk group includes patients who use medical equipment (such as catheters and endotracheal tubes), who have certain diseases (such as diabetes mellitus, chronic kidney disease, chronic heart disease), as well as those who are currently or have recently been hospitalized or undergone medical interventions [22,26]. Studies on the transmission of A. xylosoxydans were mainly local-specific, with high endemism and variability, varying from the level close to the occurrence. Intra-hospital infections are attributed to the contamination of well water, liquids (venous, hemodialysis, irrigation, mouthwash), incubators and moisturizers, as well as contaminated soap. The introduction of A. xylosoxydans through incorrectly sterilized instruments is also a frequent cause of bacteriemia. The resistance of some strains to sterilizing agents (e.g., chlorhexidine) provides a different way of infection, as they can serve as an additional reservoir[13,15,16]

The sensitivity of this bacterium to antibiotics has also been studied and consists of the following: treating Achromobacter-induced infections is a clinical problem. Internal resistance to several classes of antibiotics, mediated mainly by multi-drug outlet pumps and β-lactamases, together with acquired resistance to carbapenems caused by MBL, leaves little therapeutic possibilities for their treatment. The combination of new inhibitors β-lactam/β-lactamase with anticarapacemic activity is ineffective, as the production of serincarapacemia is not the main mechanism of *Achromobacter spp* resistance. Among other new antibiotics, erevacycline and cefiderol may play a role in the treatment of MDR Achromobacter-induced infections. Achromobacter strains are often resistant to aminoglycosides, ampicillin, first-and second-generation cephalosporins, chloramphenicol, and fluoroquinolones [2,11,17]. Achromobacter species include two well-described polypharmaceutical jet pumps and several approximate jet pump genes [27]. The AxyABM flow pump was found in all Achromobacter mass

genomes and shares common features with the flow pump MexAB-OprM P. aeruginosa [10]. AxyABM plays a significant role in the extrusion of cephalosporins, except for cefepim, cefuroxime, and aztreonam, however, this is not the only mechanism of resistance to agents, as after in vitro inhibition of AxyABM, the sensitivity to aztreonam and cephalosporin is not restored [33].

More accurate identification of species became possible thanks to the use of genotypic methods, such as nrdA gene sequencing and multicellular sequencing [8, 9]. However, for most standard clinical microbiological laboratories, sequencing identification using these methods is not available. Matrix-activated laser desorption/ionization mass spectrometry (MALDI-TOF MS) was successfully used to differentiate Achromobacter from other non-fermenting bacteria at the genus level [17, 19, 20]. Identification at the species level was difficult due to the limited number of species included in the MALDI-TOF database (for example, two and six species for Vitek MS V3.0 and MALDI Biotyper IVD-CE) (21). MALDI-TOF succeeded in accurately identifying most species (i.e. ≥90%) while expanding the database using 18 and 9 different Achromobacter species in two separate studies [21]. These studies use the standard MALDI Biotyper database.

Currently, there is no vaccine against *A. xylosoxydans*. Infection in medical institutions can be controlled by more effective preventive measures and more effective cleansing procedures.

CONCLUSION. From the search of literary data, it was proved that this type of microbe was not found in Uzbekistan and was molecular-genetically identified. There is a clear need for more clinical, microbiological, and genomic information to guide the treatment of *Achromobacter*-induced infections, which can only be obtained through multicenter research due to the rarity of these infections. Furthermore, complete genomic sequencing of isolates from the surrounding environment, in addition to clinical collections, helps to characterize the population structure and identify the mechanisms of antimicrobial resistance of Achromobacter species.

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