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Brucella and *Ochrobactrum* – differences and similarities

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Abstract

Introduction and Objective. *Brucella* and *Ochrobactrum* are genetically-related strains of bacteria belonging to the same Alphaproteobacteria class, due to their evolutionary origin. However, the two strains are distinct species. Brucella is the genus of bacteria that causes the infectious disease called brucellosis. *Ochrobactrum* is a genus of bacteria that can usually act as opportunistic pathogens with low virulence, such as Ochrobactrum anthropi. The aim of the review is analysis of available knowledge indicating similarities as well as differences between both bacteria.

Review Methods. Analysis of recent literature indicates that combining *Ochrobactrum* and *Brucella* genus causes issues, resulting in difficulties in brucellosis research and diagnosis. Distinguishing between these two types of bacteria can be time-consuming and costly, which consequently leads to delayed development of research methods, medicines, as well as vaccines against brucellosis. The following analysis draws on a publication with national and global reach.

Brief description of the state of knowledge. Currently, there is a dispute among taxonomists regarding the combination of the genus *Brucella* with *Ochrobactrum*. The idea of the combination of these two genus was based on the results of genetic analysis. However, the following assumptions do not take into account significant differences in pathogenicity, morphology, or phenotypic characteristics, which should not be ignored.

Summary. Combining the *Brucella* and *Ochrobactrum* genus may lead to misidentification, which can result in inadequate treatment or delayed diagnosis of brucellosis. In the case of brucellosis, delays in diagnosis and treatment can lead to severe complications, or even death.

Key words
Brucella, genome sequence, phenotypic, taxonomic, *Ochrobactrum*

INTRODUCTION

Ochrobactrum and *Brucella* are genetically-related bacterial genus belonging to *Brucellaceae* family in the *Alphaproteobacteria* class, with 98.8% rRNA similarity indicated. Due to their genetic relationship, the *Ochrobactrum* genus can be misidentified as the *Brucella* genus. Despite phylogenetic closeness, *Brucella* and *Ochrobactrum* are biologically different bacteria, especially in terms of their interactions with host cells. *Ochrobactrum* is a free-living environmental saprophyte with low virulence, which does not replicate in either human or animal cells. The pathogen has recently been described as the cause of disease in humans, while *Brucella* is a well-known intracellular pathogen that replicates in both human and animal cells [1, 2].

Brucellosis, due to its variable clinical presentation, is caused by *Brucella* spp. cocobacilli can be confused with other infectious or non-infectious diseases. It is essential to quickly and accurately identify *Brucella* spp. in order to provide the appropriate treatment. The identification *Brucella* genus is also crucial for epidemiological reasons, as isolating this bacterium from humans reflects its presence in the general animal populations and requires appropriate action to prevent infection outbreaks [1, 3].

The countries struggling with endemic brucellosis have to be aware of the limitations of the automated *Brucella* identification system. It is recommended to consider *Brucella* infections when an automated microbiological system detects *Ochrobactrum* spp. [1]. It is estimated that between 1.6 – 2.1 million new cases of brucellosis occur in humans worldwide each year. High incidence rates are reported in the Mediterranean region, the Middle East, Central Asia, and parts of Africa [4]. In Europe, 29 EU/EEA countries reported data on brucellosis in 2022; of these, 18 countries reported 199 confirmed cases of brucellosis. France, Germany, Greece, Italy, Portugal, and Spain reported the highest number of confirmed cases, accounting for 81% of all cases reported in the EU/EEA [5] (Tab. 1). On the other hand, data on the prevalence, incidence, and geographical distribution of *Ochrobactrum* are limited and focus on its opportunistic role in human infections, rather than on the common disease brucellosis.

Based on the latest research analysis from 2020, Hördt et al. proposed a reclassification of the species *Ochrobactrum* to the genus *Brucella*. The previous distinction between *Ochrobactrum* and *Brucella* was not based on an analysis of 16S rRNA gene or phylogenetic analyses, therefore Hördt

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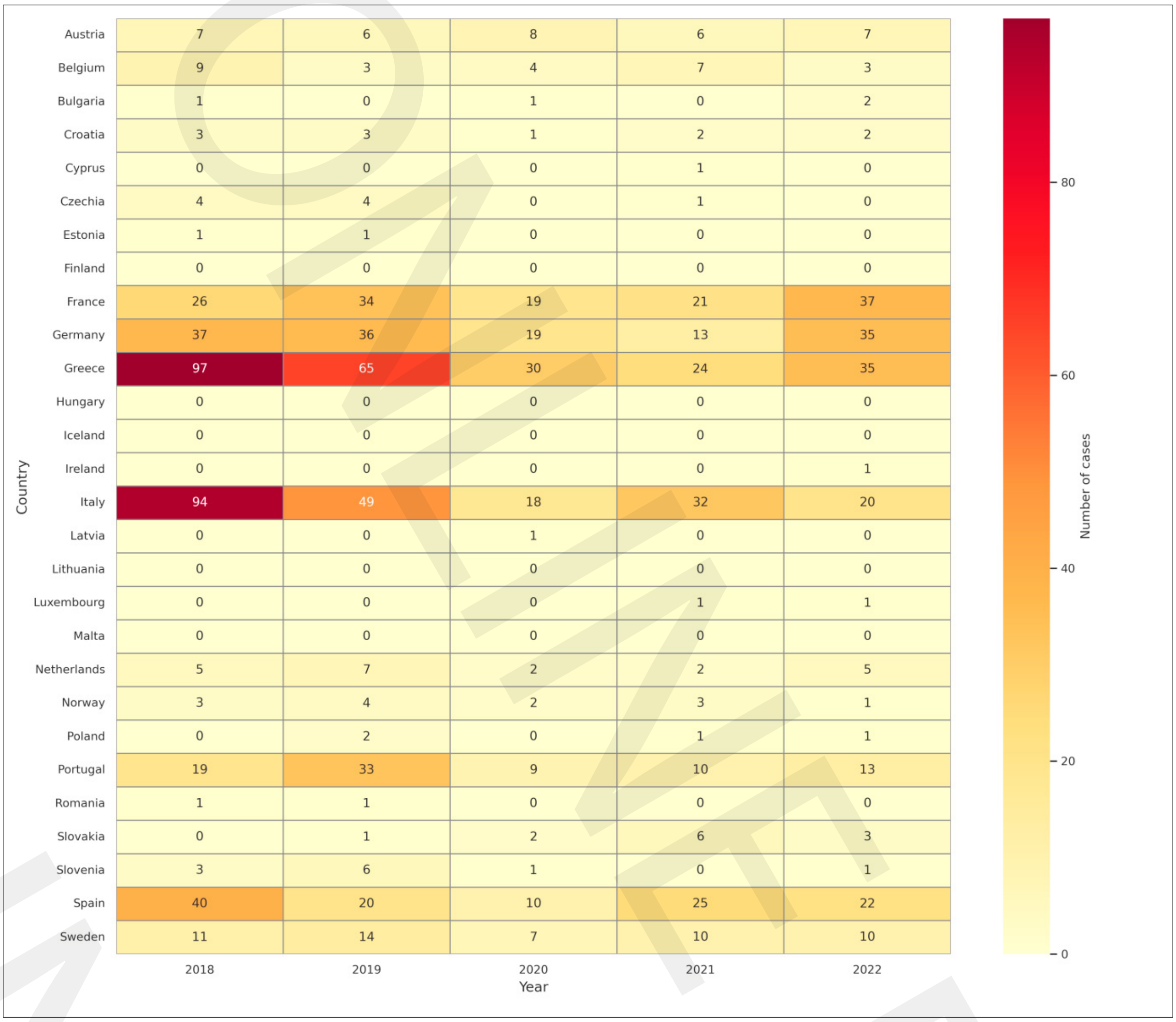
Table 1. Confirmed brucellosis cases in the EU/EEA, 2018–2022



Country	2018	2019	2020	2021	2022
Austria	7	6	8	6	7
Belgium	9	3	4	7	3
Bulgaria	1	0	1	0	2
Croatia	3	3	1	2	2
Cyprus	0	0	0	1	0
Czechia	4	4	0	1	0
Estonia	1	1	0	0	0
Finland	0	0	0	0	0
France	26	34	19	21	37
Germany	37	36	19	13	35
Greece	97	65	30	24	35
Hungary	0	0	0	0	0
Iceland	0	0	0	0	0
Ireland	0	0	0	0	1
Italy	94	49	18	32	20
Latvia	0	0	1	0	0
Lithuania	0	0	0	0	0
Luxembourg	0	0	0	1	1
Malta	0	0	0	0	0
Netherlands	5	7	2	2	5
Norway	3	4	2	3	1
Poland	0	2	0	1	1
Portugal	19	33	9	10	13
Romania	1	1	0	0	0
Slovakia	0	1	2	6	3
Slovenia	3	6	1	0	1
Spain	40	20	10	25	22
Sweden	11	14	7	10	10

Source: Annual epidemiological report for 2022. Stockholm: ECDC, 2024 [5

et al. proposed including all species of *Ochrobactrum* in the genus *Brucella*. The revised nomenclature of *Brucella* adheres to the principles set out in 2008 in the International Code of Nomenclature of Prokaryotes (ICNP) for the 18 *Ochrobactrum* species [6]. However, to distinguish the two species from the classic species, *Brucella* was named the *Brucella (Ochrobactrum)* species. Some of the identification systems, such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF), nucleic acid identification method, or automated phenotypic methods, have already implemented alternative *Brucella* designations for *O. anthropi* (currently *Brucella anthropi*), *O. intermedium* (currently *Brucella intermedia*), and others, as *Ochrobactrum* [6]. Application of the *Brucella* genus for the following organism probably also occurs in clinical laboratories.

The aforementioned measures, however, have a few consequences, the most significant of which concern are laboratories that report these organisms as *Brucella* species, but without providing any comments or information about term actualization. Such a situation can result in inappropriate treatment for brucellosis, caused by a lack of awareness among medical personnel [2, 6].

19 December 2022, the Centre for Disease Control and Prevention (CDC) published a brief laboratory report involving the actualization regarding *Brucella/Ochrobactrum* strains via the Laboratory Outreach Communication System – 'Reclassification of *Ochrobactrum* species into the *Brucella* genus'. The update aimed to assist laboratories in solving identification issues with the organism classified as *Brucella* in the class II biosafety level. Furthermore, it is noted that all bacterial isolates presumptively identified as Brucella species should be reported to the relevant State public health laboratory for additional testing. Taking into account reclassification, the laboratory needs to take further actions and guidelines relating to distinguishing the species of the selected factor *Brucella* from the non-selected factor *Brucella* (*Ochrobactrum*), and to clarify the reporting roles of these organisms [6]. Examination of the *Ochrobactrum* strains extracted from human clinical samples indicates that the G+C content was between 56 – 59 mol%. What is more, the DNA-rRNA hybridization parameters indicate that *Ochrobactrum* includes the branch of rRNA *Brucella* within the superfamily rRNA IV. Above the genus taxonomic level, *Ochrobactrum* is closely related to *Brucella*, *Phyllobacterium, Rhizobium,* and *Agrobacterium*. The representative species is *Ochrobactrum anthropi* [7].

REVIEW METHODS

The literature search was conducted using the Google Scholar, Scopus, and PubMed databases and covered publication during 2015 – 2025. Publications in both Polish and English were included in the analysis. Key words used in the search included *Brucella*, *Ochrobactrum*, taxonomy, phylogeny, phenotypic characteristics, pathogenicity, and antibiotic resistance. The search included original articles, review articles, reports, epidemiological data, and announcements from public health institutions, such as ECDC and the WHO. A total of over 37,000 publications were retrieved (Fig. 1).

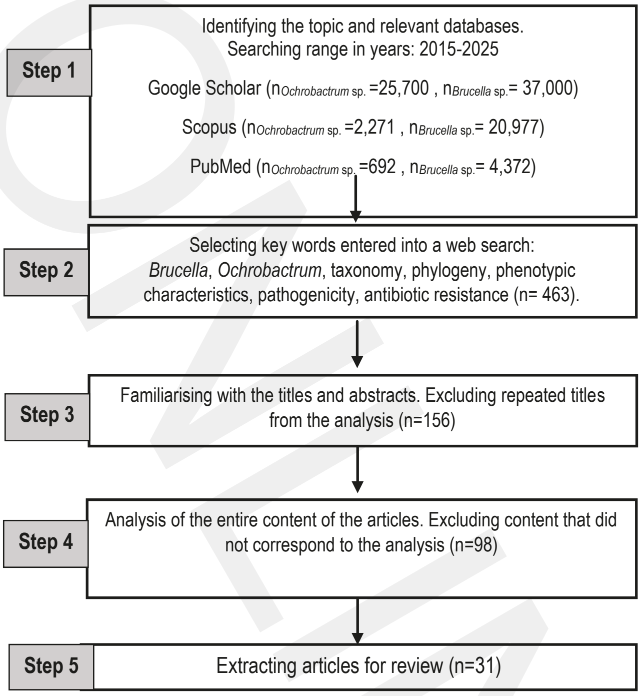


Figure 1. Review methods – algorithm of the process

***Ochrobactrum* characteristics.** *Ochrobactrum* is a Gram-negative, lactose non-fermenting, and low virulence rod, with which the *Brucella* genus is a close neighbour. The *Ochrobactrum* genus belongs to the family *Brucellaceae* and is included in the class *Alfaproteobacteria*. The bacterium was first described by Holmes et al. in 1988 as a microorganism most closely related to the *Brucella* genus, in which many pathogenic species to humans and animals belongs. Since the establishment of the *Ochrobactrum* genus in 1988, several distinct species have been characterized and recognized as opportunistic pathogens in various disease outbreaks [8, 9]. The review conducted by Ryan and Pembroke identified 128 species of *Ochrobactrum* spp., which are increasingly responsible for severe infections. Infection review programmes should therefore include an investigation plan for possible *Ochrobactrum* spp. outbreaks if the bacteria have been isolated in more than one patient. *Ochrobactrum* spp. occurs in various environmental conditions, such as water, soil, plants, or animals. Several species have been studied for the degradation of xenobiotic pollutants and detoxification of heavy metals under different environmental conditions. Although they are considered to be relatively non-virulent, they are increasingly being found to cause infection, certain severe conditions, including endocarditis and septicemia in hosts with reduced immunity [8, 10]. Research in the scientific and medical literature revealed a wide range of infections caused by *Ochrobactrum* spp. resistant to an extensive range of antibiotics [8]. The data indicate that the species *Ochrobactrum* spp. is a pathogen that occurs more often than previously thought and can cause many more infections or medical conditions, which suggests that it is an aggressive and devastating microbe [8, 9]. Currently, thirteen species of *Ochrobactrum* have been described: *O. anthropi, O. intermedium, O. tritici, O. grignonense, O. gallinifaecis, O. lupini, O. oryzae, O. cytisi, O. pseudogrignonense, O. haematophilum, O. pseudointermedium, O. rhizosphaerae,* and *O. thiophenivorans.* In 2007, the genome of *O. anthropi* ATCC 49188 was sequenced. The genome consists of two chromosomes (2,9 Mbp and 1,9 Mbp) and 4 megaplasmids (pOANTOl, pOANT02, pOANT03, pOANT04). Bacteria of the *O. anthropi* species have been characterized as human opportunistic pathogens, as they were most often isolated from immunocompromised patients with symptoms of bacteremia. Between 1998 – 2008, similar origins and properties of the bacteria were described and belonged to the species: *O. intermedium, O. pseudointermedium, O. pseudogrignonense, and O. haematophilum* [11]. In recent years, there have also been reports of the isolation of the *Ochrobactrum* genus from the papillae of legumes (formerly legumes) and rice, where they play an important role in fixing atmospheric nitrogen. Furthermore, a range of isolates belonging to the *Ochrobactrum* genus obtained from a variety of environments (rivers, canals and sewage, cultivated soil, rhizosphere) [9], often contaminated with products of human management. The ability of these bacteria to colonize diverse environments, especially those with high levels of pollution from industrial sewage, pesticides, and herbicides, has attracted research interest [12]. *Ochrobactrum* infection is often hospital-acquired and occurs in patients with permanently installed medical devices and implants. The most common *Ochrobactrum* species that infect humans are: *O. anthropi*, *O. intermedium,* and *O. pseudintermedium* [13].

Subramanian et al. presented a case of septicaemia caused by *Ochrobactrum intermedium* in a 75-year-old patient with cellulitis of the lower extremities, in which the epidemiology, clinical symptoms, laboratory diagnosis, antibiotic sensitivity, and treatment of the infection were described [11]. *Ochrobactrum* is phenotypically very similar to the *Brucella* species, and laboratory misidentification is not uncommon. Although bacteria belonging to this genus have low virulence, infection in patients with diseases and lowered immunity can be serious [13]. *Ochrobactrum anthropi* is the most common species of the genus *Ochrobactrum*. *Ochrobactrum intermedium* is a new multidrug-resistant pathogen whose clinical features are poorly characterized.

Another case reported in India was that of a 75-year-old man suffering from diabetes and hypertension, diagnosed with septicaemia caused by *Ochrobactrum intermedium.* *Ochrobactrum* species are resistant to all beta-lactams except carbapenems due to the presence of AmpC beta-lactamases, and are usually sensitive to aminoglycosides, trimethoprim-sulfamethoxazole, rifampicin, and fluoroquinolones [13]. The antibiotic susceptibility of the isolate from the septicaemia patient followed the susceptibility pattern of previously described *Ochrobactrum* isolates from human infection, except that they showed intermediate susceptibility to aminoglycosides (amikacin and gentamicin). *Ochrobactrum intermedium* indicates resistance to a wide range of antibiotics (beta-lactams, aminoglycosides, and colistin) compared to *Ochrobactrum anthropi*. Colistin resistance, a negative urease test, and growth on culture medium are essential phenotypic tests to distinguish *O. intermedium* from *O. anthropi*. In the current case, late diagnosis, the presence of co-existing diseases, antibiotic resistance, and the age of the patient, were all factors that could potentially have led to death [11]. Although the isolate showed relatively rapid growth on MacConkey agar, which is rather incompatible with *Brucella* identification, several additional differential tests were performed to exclude the possibility of Brucella, for which phenotypic tests were performed. The isolate was motile and utilised mannitol, sorbitol, and D-arabitol. Based on the 16S rRNA sequencing using NCBI BLAST (Basic Local Alignment Search Tool), the isolate was identified as *Ochrobactrum intermedium* (GenBank Access No. OM302143) with similarity of sequence > 98.65% [9]. *Ochrobactrum* species can also be part of the normal flora of our large intestine.

Although the *Ochrobactrum* species are recognized as opportunistic human pathogens with low virulence, most of the reported cases involve *O. anthropi*, which is usually associated with intravenous catheterization infection and often occurs in immunocompromised patients [9, 13, 14]. On the other hand, *O. intermedium*, a new human pathogen that cannot be easily distinguished from other *Ochrobactrum* species using conventional methods, is rarely described in the literature. *Ochrobactrum intermedium* can form microabscesses and is known for its multi-drug resistance. An example is the case of an 84-year-old patient diagnosed with cholangitis caused by *O. intermedium* infection, and successfully treated with minocycline. Even though *O. intermedium* rarely, the differential diagnostics should be considered in patients with biliary and intestinal pathology, especially in immunocompromised patients [14].

***Brucella* characteristics.** *Brucella* is a gram-negative coccbacilli, an intracellular pathogen which is particularly dangerous for domestic animals, in which it can cause an infectious disease called brucellosis. Infection causes abortions and infertility in such domestic animals as sheep, rams, and pigs. Species of serious concern for humans are: *Brucella melitensis, Brucella suis, Brucella abortus,* and *Brucella canis*, which cause non-specific flu-like symptoms [3, 15]. Microorganisms of the genus Brucella are classified in the class *Alphaproteobacteria,* belonging to the family *Brucellaceae*. Currently, the genus *Brucella* includes twelve species of facultative intracellular bacteria with variable zoonotic potential [3]. Six of these were found to cause classic diseases, causing terrestrial mammalian brucellosis, while two species originated from marine mammals.

Over the past fifteen years, field studies and improved pathogen detection have enabled the identification of four new species: *Brucella microti, Brucella inopinata, Brucella papionis,* and *Brucella vulpis,* as well as numerous strain isolates from a wide range of animals, including, for the first time, ectothermic animals. Even though their genome sequences are still very similar to those of classical strains, some exhibit atypical phenotypes, including a greater growth rate, increased resistance to acid stress, motility, and mortality in a mouse model of infection [16]. *Brucella* rods introduced by host cells, through inhalation, skin abrasions, ingestion, or mucous membranes, can survive and multiply in both phagocytic and non-phagocytic cells. These bacteria do not produce the classic virulence factors, such as exotoxins, cytolysins, exoenzymes, plasmids, fimbriae, and drug-resistant forms. The main virulence factors of *Brucella* are lipopolysaccharide (LPS), the T4SS secretory system, and the BvrR/BvrS system, which allow interaction with the surface of the host cell, formation of early and late BCV (Brucella-containing vacuole), and interaction with the endoplasmic reticulum (ER) during bacterial proliferation [2, 3].

Brucella is a pathogen that causes massive infection, resulting in significant economic losses. Moreover, people who work with infected animals, such as farmers, veterinarians, and laboratory technicians, and are among those most likely to come into contact with the pathogen and therefore among those at risk. Brucellosis in humans causes non-specific symptoms, thus rendering the number of infected people to be accurately estimated. The process of infection is also complex, involving many unexplained issues, which necessitates further research into the mechanisms of infection [3, 15]. The *Brucella* genus belongs to the *α-2* subdivision of *Proteobacteria,* which includes plant and animal pathogens associated pericellularly or intracellularly with plant cells (*Agrobacterium* and *Rhizobiaceae*), as well as intracellular mammalian pathogens (*Brucella, Bartonella, Ochrobactrum,* and *Rickettsiae*). The official classification is based solely on phenotypic characteristics identified through numerous bacteriological and biochemical tests. Following the classical nomenclature, six species were initially distinguished based on their preferred host, their susceptibility to lysis by multiple specific phages, and their pattern of oxidising various carbohydrate and amino acid substrates. In recent years, the number of identified species increased to ten after the isolation of several new species from marine mammals, voles, and rodents, as well as infected human breast implants.

Brucella melitensis, Brucella abortus, and *Brucella suis* are three species commonly associated with human diseases [3, 17]. Rare cases of human infection were reported for *Brucella canis,* whereas no human cases of infection were reported for *Brucella ovis* and *Brucella neotomae*. Furthermore, one possible laboratory-acquired infection with a marine mammal isolate was presented, and one specific sequence type (ST27) was linked to three human infections in Peru and New Zealand, where the patients had no contact with marine mammals, but contact with raw fish was a common feature of the three cases. In Egypt, a report of *B. melitensis* bv2 detection in catfish suggests that fish may be another source of infection [17].

The intracellular pathogens of the genus *Brucella* are phylogenetically similar to *Ochrobactrum*, a diverse group of free-living bacteria. A group of taxonomists included all *Ochrobactrum* organisms in the genus *Brucella,* based on global genome analyses and likely equivalencies with genus such as Mycobacterium. Moreno et al. indicated that such equivalencies are incorrect, due to the excluded complexity of pathogenicity. Divergences in *Brucella* and *Ochrobactrum* lifestyle, structure, physiology, population, genomic features, and pathogenicity, indicate their significant importance in taxonomy. Epidemiology, prevention, diagnosis, and treatment are completely independent, which is why the combination of free-living *Ochrobactrum* with highly

Table 2. Differences between *Brucella* and *Ochrobactrum*

Feature	*Ochrobactrum* [1, 6, 8]	*Brucella* [1, 4, 6, 8]
Classification	Family *Brucellaceae*, class *Alphaproteobacteria*	Family *Brucellaceae*, class *Alphaproteobacteria*
Relatedness	~98.8% 16S rRNA similarity with *Brucella*	Closely related to *Ochrobactrum*
Environment	Widely distributed: soil, water, plants, animals; often saprophytes	Pathogens of terrestrial and marine mammals, no free-living reservoirs
Pathogenicity	Opportunistic pathogens, low virulence; infections mainly in immunocompromised individuals (e.g., *O. anthropi*, *O. intermedium*)	Highly pathogenic, pathogenic to animals and humans – causes brucellosis
Lifestyle	Free-living, extracellular; does not replicate in host cells	Intracellular pathogens capable of surviving and multiplying in phagocytes
Morphology	Gram-negative coccobacilli pleomorphic, larger cells	Gram-negative coccobacilli, smaller and often poorly staining
Growth on media	Rapid growth. Blood and chocolate agars: >0.5 mm after 24 hr, >0.5 mm after 48–72 hr. MacConkey agar: >0.5 mm after 24 hr (delayed growth on MacConkey agar observed in some isolates of Brucella (Ochrobactrum) species), non-lactose fermenting, >0.5 mm after 48–72 hr. white/transparent, slimy colonies	Slower growth, smooth, creamy colonies, do not grow on MacConkey agar
Virulence factors	Lack of classical factors, frequent antibiotic resistance (AmpC β-lactamases)	LPS, T4SS secretion system, BvrR/BvrS cell survival system
Antibiotic resistance	Natural resistance to most β-lactams (except carbapenems), sensitivity to fluoroquinolones, TMP-SMX, and aminoglycosides	Does not exhibit such broad natural resistance; therapy is difficult due to intracellular survival
Treatment therapy	Imipenem, fluoroquinolones, TMP-SMX, aminoglycosides; no established regimen	Long-term combination therapy: doxycycline + rifampicin (6–8 weeks), alternatively doxycycline + streptomycin
Routes of infection	Hospital-acquired infections, most often related to catheters, implants, and medical devices	Contact with animals, animal products (unpasteurized milk, meat), inhalation, damaged skin
Epidemiological significance	Increasingly isolated as a cause of opportunistic infections, little importance in public health	A serious zoonosis worldwide; great epidemiological and economic importance
Environmental use	Participation in the biodegradation of pollutants, nitrogen fixation in plants	No environmental properties, obligate pathogens

pathogenic *Brucella*. The following situations increase the risk for veterinarians, medical doctors, and public health authorities who are faced with a common, worldwide zoonosis such as brucellosis. Therefore, from a taxonomic and practical point of view, the *Brucella* and *Ochrobactrum* genus must be considered separately [18].

Phenotypic differences between *Ochrobactrum* and *Brucella* and treatment of infections. *Brucella spp.* and *Ochrobactrum spp.* show significant differences in culture, both in growth rate and colony morphology. *Brucella* grows relatively more slowly on culture media than *Ochrobactrum*, with a characteristic appearance of colonies that are smooth, creamy, and non-haemolytic, whereas *Ochrobactrum* grows faster and forms white or transparent colonies, also non-haemolytic, but with a different texture [6, 20]. Additionally, differences in cell morphology are clearly visible under the microscope – *Brucella* colonies are smaller and have a more distinct appearance than *Ochrobactrum,* they also differ in cell shape – Brucella cells are coccobacilli, whereas *Ochrobactrum* cells are more elongated [3, 6, 19]. *Ochrobactrum* bacteria, unlike *Brucella* species, are characterized by rapid colony growth on MacConkey agar (>0.5 mm after 24 hours) and have a mucoid colony morphology [10]. Classic *Brucella* species, such as *B. melitensis*, are small, often poorly staining, Gram-negative rods that are susceptible to discolouration and may appear to be Gram-positive. Cells of the *Ochrobactrum* species exhibit pleomorphism – the phenomenon of having different shapes of cells and cell organelles, or form rod-shaped cells that are larger than those of selected *Brucella* species [6, 20]. The outer membrane of *Ochrobactrum* and *Brucella* also differs. The membrane of *Brucella* is highly permeable to hydrophobic substances, whereas the membrane of *Ochrobactrum* is not, leading to different patterns of susceptibility. The bacterial outer cell membrane component – lipopolysaccharide LPS in *Brucella* exhibits charge reduction, resulting in intrinsic resistance to polymyxins, whereas *Ochrobactrum anthropi* is susceptible to polymyxins and is associated with sporadic cases of endocarditis, bacteraemia, post-operative and nosocomial infections, mainly in immunocompromised patients. There is no treatment for *O. anthropi* infection [1, 9].

Disease in human caused by the classical *Brucella* species is often observed in those working with animals or in contact with animal meat, people eating unpasteurized animal products (dairy products) contaminated with *Brucella* [19], or people who have travelled to regions where these products are available, and those who have had contact with wildlife in *Brucella*-endemic areas. The *Ochrobactrum* species, which occur in/on water, soil, plants, and animals, due to their low virulence usually cause infections in immunocompromised individuals. Hospital infections caused by *Ochrobactrum* most often have an invasive origin from contaminated equipment [6, 20]. Differences between *Brucella* and *Ochrobactrum* also occur in antimicrobial therapy (AST – Antimicrobial Susceptibility Testing). For the treatment of brucellosis, combination treatment with doxycycline and rifampicin for at least 6 – 8 weeks is recommended. Alternatively, doxycycline and streptomycin are recommended, along with serological monitoring for more than 24 weeks after laboratory exposure to the causative agent of brucellosis [2]. Infection caused by the *Brucella* species (*Ochrobactrum*) is effectively treated with imipenem, fluoroquinolones, trimethoprim-sulfamethoxazole, or aminoglycosides [3, 10, 21] (Tab. 2).

Taxonomic and phylogenetic analysis of *Brucella* and *Ochrobactrum*. Given the great confusion surrounding

Ochrobactrum and *Brucella strains*, She et al. compiled a list of all known *Ochrobactrum 'Brucella'* species, noting the difficulties in identifying the 'true' *Brucella* strain when using certain MALDI-TOF MS spectrometry methods, nucleic acid detection methods, and automated databases [6]. The use of simple tests designed to distinguish between these different bacteria also illustrates the questionable basis of this association. *Brucella* and brucellosis, regardless of species, cause the same set of symptoms, differing in virulence and host preferences. The combination of *Ochrobactrum* and *Brucella* began without the involvement of brucellosis and *Ochrobactrum* experts. The taxonomic approach provides names for living organisms, while phylogeny studies the evolutionary history. Taxonomy should be applied in a group of bacteria by responsible, consensual agreement among experts and stakeholders, especially in the case of dangerous pathogens. Taxonomic names can have serious consequences if they are not adapted to the facts of microbiology. Therefore, taxonomy should be a system from which meaningful information can be obtained, and not a cumbersome system of names detached from reality [22, 23]. An interesting study by Massot et al. analysed the P6BS-III pangenome, and contributed to a better understanding of the *Ochrobactrum* genus. Genome sequence analysis of the *Ochrobactrum haematophilum* strain P6BS-III (DSM 106071), isolated from pastures in Buenos Aires Province, Argentina, exposed to the herbicide glyphosate, indicated tolerance to glyphosate, combined with the expression of plant growth, and proved that this strain is a potential modifier of agriculturally valuable plant species in phytoremediation [24]. Intracellular pathogens of the genus *Brucella* are phylogenetically related to *Ochrobactrum*, a diverse group of free-living bacteria with several species occasionally infecting patients. A group of taxonomists has included all *Ochrobactrum* organisms in the genus *Brucella* based on global genome analyses. Moreno et al. have shown that such equivalences are incorrect because they ignore the complexity of pathogenicity. The discrepancies in the lifestyle of *Brucella* and *Ochrobactrum*, structure, physiology, population, genomic features, and pathogenicity are very important in taxonomy. Therefore, the differences between *Ochrobactrum* and *Brucella* are not limited to their assignment to different 'risk groups'. Therefore, from a taxonomic and practical point of view, *Brucella* and the genus *Ochrobactrum* must be considered separately [18].

Yang et al. studied blood and bone marrow samples from the injured left tibia obtained from two patients with fever without an apparent cause. The specimens were initially identified as *Bordetella bronchiseptica* (Case 1) and *Ochrobactrum anthropi* (Case 2) using the Vitek 2 Compact microbial identification system with a Gram-negative identification card. In the first case, where the pathogen was misidentified as *Bordetella bronchiseptica*, the patient had brucellosis and showed typical symptoms of splenomegaly, mildly elevated aminotransferase activity, fluctuating fever, and significantly elevated lymphocyte counts. PCR amplification of 16S rRNA and sequencing were performed and the results analyzed using GenBank. The results showed that *Brucella abortus, Brucella melitensis and Brucella microti, Brucella spp.*, as well as *Ochrobactrum anthropi* ATCC 49188, had the highest level of identity [25]. After conducting the assay using real-time PCR, melting curves were analyzed to identify *Brucella strains*. Due to repeated misidentifications, more and more laboratories are now relying on molecular methods to identify *Brucella*. In contrast, the 16S rRNA gene sequencing method is an effective clinical method for identifying free-ranging Gram-negative rods, although there are serious concerns related to the complete lack of homology with *Ochrobactrum anthropi* compared to 99% identity with *Brucella spp.* or the *Ochrobactrum anthropi* strain (including the ATCC strain) described in the publication [25]. Several studies described by Vizcaíno et al. have shown that the genus *Brucella* is closely related to members of the α-2 subclass of *Proteobacteria*, as reflected in phylogenetic trees. *O. anthropi* was found to be most closely related to *Brucella,* and two strains of *O. anthropi* were closer to the genus *Brucella* than to *Ochrobactrum,* giving rise to the proposal of a new species in the genus *Ochrobactrum – O. intermedium sp.* Interestingly, many members of the α-2 subclass of the class *Proteobacteria* live in close association with eukaryotic cells, either as pathogens or as symbionts (e.g., *Brucella, Rhizobium, Agrobacterium, Ochrobactrum, Bartonella*). It has therefore been proposed that the divergence from a common ancestor was influenced by the timing of the emergence of their respective eukaryotic hosts, and that the common ancestor of *Brucella species* may have diverged from the ancestor of the related human pathogenic bacterium *O. anthropi* between 180 and 75 million years ago, a period marked by the emergence of mammals and ungulates. A high level of homology between *Brucella* species has been demonstrated by DNA-DNA hybridization and 16S rRNA sequencing [26]. The closest neighbours of the *Brucella* microorganism are saprophytic bacteria living in soil, in particular members of the developing genus *Ochrobactrum, Pseudochrobactrum, Paenochrobactrum,* and *Falsochrobactrum* [27]. It has been proposed that species of the genus *Ochrobactrum* be reclassified as species of the genus *Brucella,* based on whole genome comparisons [28]. However, existing and proposed *Brucella species* identified earlier, still constitute a monophyletic group within any expanded genus. Although brucellosis has been eradicated in some of the more developed regions of the world, it remains a serious problem for animal and human health in many areas outside Northern Europe, North America, and Australasia.

Whatmore et al. reviewe how molecular approaches have begun to contribute to the emerging understanding of the genetic diversity of the group and the implications for taxonomy, typing, and potential applications in molecular epidemiology [29]. The current update of the review focuses on advances resulting from PCR-based techniques and the use of automated DNA sequencing approaches [30]. The identification of new *Brucella species* of minor zoonotic importance has opened a debate on the best taxonomic approach to the classification of *Brucellaceae,* namely, whether the genus *Ochrobactrum* should be included among the *Brucella*-like organisms or whether the genus *Brucella* should include atypical strains. Early in the history of brucellosis, the minimal standards that best described *Brucella* were based on the affiliation of a strain to its natural host, unique metabolic profiles, and susceptibility to phages. These features were sufficient to divide the genus *Brucella* into two groups: 'classical *Brucella*' with a common genome and phenotype, and atypical species with diverse phenotypic and genomic properties. Among the classical *Brucella, B. melitensis, B. abortus*, and *B. suis* are the three most dangerous human pathogens that threaten domestic animals associated with meat and dairy production. Therefore, the control and prevention of zoonotic brucellosis is of great public interest in most developing countries [3, 31].

CONCLUSIONS

The inclusion of *Ochrobactrum* spp. in the genus *Brucella* spp. lead to misdiagnosis, incorrect treatment, and inappropriate prophylaxis in preventing infection. *Brucella* requires a specific approach to treatment and prophylaxis, which cannot be applied to infections caused by *Ochrobactrum*, which are more often associated with opportunistic infections. A group of taxonomists has grouped *Brucella* with the free-living, phylogenetically related *Ochrobactrum spp.* According to the taxonomists, the combination of these two species is based mainly on a two-dimensional genomic analysis (at the level of sequence divergence) using a cladistic evolutionary 'concept' of the genus, rather than on an in-depth phylogenetic analysis that would take into account biologically significant characters. Furthermore, the decision to combine these species was made without the input of experts on brucellosis and *Ochrobactrum*, which is particularly worrying given the public health importance of brucellosis. Additionally, combining these two species does not take into account the significant differences between them in terms of structure, metabolism, physiology, population structure, genome, genomic features, clinical manifestations, treatment, diagnosis, and most importantly – pathogenicity. Combining these two genus into one would risk confusion and harm to veterinarians, physicians, clinical laboratories, health authorities and regulators dealing with brucellosis, particularly in low- and middle-income countries, would complicate the diagnosis, treatment and prevention of brucellosis, and would lead to the erroneous belief that all species belonging to the combined genus pose the same risk to public health

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