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If You're Not Confused, You're Not Paying Attention

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If You're Not Confused, You're Not Paying Attention: *Ochrobactrum* Is Not *Brucella*

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ABSTRACT Bacteria of the genus *Brucella* are facultative intracellular parasites that cause brucellosis, a severe animal and human disease. Recently, a group of taxonomists merged the brucellae with the primarily free-living, phylogenetically related *Ochrobactrum* spp. in the genus *Brucella*. This change, founded only on global genomic analysis and the fortuitous isolation of some opportunistic *Ochrobactrum* spp. from medically compromised patients, has been automatically included in culture collections and databases. We argue that clinical and environmental microbiologists should not accept this nomenclature, and we advise against its use because (i) it was presented without in-depth phylogenetic analyses and did not consider alternative taxonomic solutions; (ii) it was launched without the input of experts in brucellosis or *Ochrobactrum*; (iii) it applies a non-consensus genus concept that disregards taxonomically relevant differences in structure, physiology, population structure, core-pangenome assemblies, genome structure, genomic traits, clinical features, treatment, prevention, diagnosis, genus description rules, and, above all, pathogenicity; and (iv) placing these two bacterial groups in the same genus creates risks for veterinarians, medical doctors, clinical laboratories, health authorities, and legislators who deal with brucellosis, a disease that is particularly relevant in low- and middle-income countries. Based on all this information, we urge microbiologists, bacterial collections, genomic databases, journals, and public health boards to keep the *Brucella* and *Ochrobactrum* genera separate to avoid further bewilderment and harm.

KEYWORDS *Brucella*, *Ochrobactrum*

Names of infectious diseases and their etiological agents are relevant because they describe the properties of these entities and thus are essential medical and veterinary terminologies. For example, tuberculosis, brucellosis, tetanus, and gonorrhea are terms that

TABLE 1 Concerns arising from the *Ochrobactrum/Brucella* cladistics presented in reference 1

Concern	Comment
Phylogeny based on evolutionary abstraction	The authors extol the utility of alignment supermatrices of core genomes for inferring trees of closely related species, but they infer a tree based on BLASTp distance neighbor joining with minimum evolution refinement for all alphaproteobacteria and then, with this tree, revise the closely related <i>Brucella/Ochrobactrum</i> , whose core genomes are easily alignable.
Phylogenetics without context	The omission and addition of sequences commonly change tree topologies. Leaving out samples that are not type strains will likely necessitate further revisions to correct misleading topologies or to account for yet-to-be type strain placements.
The proposed " <i>Brucella</i> " is polyphyletic at conception	All brucellae (core and not core) are consistently recovered as monophyletic; however, <i>Ochrobactrum</i> is commonly rendered polyphyletic by <i>Brucella</i> but also <i>Pseudochrobactrum</i> , <i>Falsochrobactrum</i> , and <i>Paenochrobactrum</i> , as shown in the authors' own rRNA tree and the works they cite.
Omission of alternative taxonomy fixes	Renaming the <i>Ochrobactrum thiophenivorans</i> clade to another genus resolves the polyphyly presented by the authors, keeping <i>Brucella</i> and the <i>Ochrobactrum anthropii/intermedium</i> clade monophyletic. This resolution is supported by Leclercq et al. (19) and the GTDB (https://gtdb.ecogenomic.org) and leaves the type species of <i>Ochrobactrum</i> (<i>Ochrobactrum anthropi</i>) and <i>Brucella</i> (<i>Brucella melitensis</i>) within their respective genera.

have been unequivocally linked to particular bacterial pathogens for over one century. For those who understand their meaning, these names are not vague concepts but rather precise medical conditions that require treatment and prevention. While cephalosporins are recommended to combat *Neisseria gonorrhoeae*, the causative agent of gonorrhea, this antibiotic does not cure tuberculosis or brucellosis caused by *Mycobacterium* or *Brucella* organisms, respectively. Similarly, vaccines that prevent a specific infection do not protect against other bacterial diseases.

In this context, the need for different prevention and treatment strategies exemplifies the profound differences among pathogens and their biological diversity. For medical practitioners and veterinarians, using names molded by scientific interactions with microorganisms for over one century is not a professional onomatomania but a triumph in understanding complex processes and a serious responsibility. For this reason, introducing or modifying nomenclatures should be done with the cooperation of experts and consensus on the subject. Otherwise, there is a risk of causing confusion and damage rather than clarity and benefit.

Particularly problematic has been a publication by bacterial taxonomists who included *Ochrobactrum* within the genus *Brucella* (1), a nomenclature recently examined in the *Journal of Clinical Microbiology*, albeit not without warning (2). As widely known, the brucellae are dangerous intracellular pathogens of animals and humans, while *Ochrobactrum* organisms are free-living organisms associated with soil and plants. Those taxonomists justified such merging based on a two-dimensional genomic analysis (chiefly, the level of sequence divergence) and applied a cladistic rather than systematic evolutionary "concept" of the genus (see reference 3 for a discussion). However, only the latter aligns with the polyphasic taxonomy recommended in authoritative prokaryotic taxonomy manuals, because it includes both genomic analyses and biologically significant traits (4). Consistent with their perspective on genus definition, those taxonomists attempted to minimize the differences by arguing that these phylogenetically related bacteria are not markedly separated because they merely belong to two different "risk groups" and "*Ochrobactrum* species are also known from clinical specimens" (1).

Aside from the lack of appropriate in-depth phylogenetic analyses (Table 1) and discussions of other phylogenetic hypotheses and alternative taxonomic solutions (all without the necessary *Brucella/Ochrobactrum* expert input), the proposal was refuted on the basis of relevant characteristics (3). These characteristics include divergent lifestyles and differences in structure, metabolism, physiology, population structure, core-pangenome assemblies, genome structure, genomic traits, clinical features, treatment, diagnosis, and, above all, pathogenicity and risk groups (Table 2), arguments taxonomically more relevant than a limited phylogenetic analysis alone. These differences make unfeasible a biologically meaningful

TABLE 2 Comparison between the *Brucella* and *Ochrobactrum* genera^a

Finding for:		<i>Brucella</i>	<i>Ochrobactrum</i>
Divergent property		<i>Brucella</i>	
Genome size (Mb)		3.1–3.4	4.7–8.3
Pangenome type (no. of genes)		Closed (~11,000)	Open (>74,000)
No. of genes in core genome		~1,000	~75
DNA-DNA hybridization (%)		~20–30 ^b	
No. of RNA genes		54	78–85
Presence of IS711 insertion sequences		In all species and strains	Absent
No. and type of plasmids		None	Variable (up to 6) and conjugative
Phylogeny		Monophyletic	Polyphyletic
No. of lysogenic phages		None	>4 (present in at least in some species)
Lateral gene transfer		Absent	Present
Speciation type		Allopatric	Sympatric
Overall cell envelope properties		Permeable to hydrophobic probes and resistant to polycationic peptides	Impermeable to hydrophobic probes and sensitive to polycationic peptides (<i>O. anthropi</i> and <i>O. intermedium</i>)
No. of transport reactions		~47	~111
Metabolic redundancy		Low	High
No. of metabolic pathways		254 (35 unique for the genus)	313 (94 unique for the genus)
Removal of toxic metals		No	Yes (species/strains)
Degradation of phenolic compounds, petroleum wastes, and xenobiotics		No	Yes (species/strains)
Capable of root nodulation		No	Yes (species/strains)
Lifestyle		Pathogen	Saprophyte
Natural habitat		Intracellular	Soil and root plant surfaces
Transmission to humans		Host-host interaction/animal products	Mostly iatrogenic ^b
Virulence		Finely tuned	Fortuitous/opportunistic ^b
Virulence mechanisms		Escape from the immune response/deviation of intracellular trafficking	No true virulence mechanisms (virulence depends on the host's immune status)
Type IV secretion system		Required for intracellular trafficking and lifestyle	Devoted to plasmid conjugation with a different origin
Infection dynamics		Long-lasting infection and low proinflammatory response	Acute proinflammatory/pyrogenic; self-limiting in immunocompetent hosts ^b
Animal disease		Globally distributed and prioritized in many countries (20); about 1.25 billion and 1.9 billion susceptible cattle and small ruminants, respectively, in nonindustrialized countries (with endemic disease) ^c	Not reported as agents of contagious disease
Human health		Present in at least 101 countries worldwide (in 2018) and hugely underreported (21); based on fragmentary (but valid) seroprevalence studies, the annual number of cases may be in the range of 330,000–19,000,000 cases (22)	A total of 288 cases published between 1998 and 2020 (9)
Diagnosis		Well-standardized serological methods	No serological tests are available or necessary
Treatment		WHO-recommended long bithérapie in uncomplicated cases (doxycycline and streptomycin or doxycycline and rifampin)	Depending on antibiotic resistance, short-term broad-spectrum intravenous monotherapy with β -lactams, such as imipenem-clastatin or cefepime, or oral therapy with co-trimoxazole or ciprofloxacin (9)
Acquired antibiotic resistance		Not reported for doxycycline and streptomycin, including isolates from relapse cases; reported rifampicin resistance in a few strains, possibly due to <i>in vitro</i> overestimation (23), and very rarely confirmed by <i>ropB</i> mutations (24)	Plasmid-encoded resistance to different families of antibiotics, such as β -lactams (penicillins and cephalosporins, with emerging cases of carbapenem resistance) (9)

(Continued on next page)

TABLE 2 (Continued)

Divergent property	Finding for:	
	<i>Brucella</i>	<i>Ochrobactrum</i>
Vaccine	Available (for domestic ruminants) and critically important to control disease	Not available or recommended
WHO/OIE/FAO recommendations/regulations	Detailed, as follows: (i) humans: diagnosis, treatment, and prophylaxis; (ii) animals: diagnostic procedures/protocols (with emphasis on prescribed tests for international trade) and vaccination	None
WHO biosafety risk group (human disease)	RG3 (high individual risk and low community risk)	RG1 (no or low individual and community risk)

^aData extracted from reference 3 (copyright owned by the authors).

^bCompared with the *O. anthropi/O. intermedium* group, the closest phylogenetic relatives of *Brucella* species (6).

^cFigures were calculated by totaling FAOSTAT population data for 2013 for the European Union, North America, Australia, and New Zealand (industrialized countries) and subtracting this value from the world sheep and goat population (<https://www.fao.org/faostat/es/#data> [accessed 6 June 2023]).

description of the expanded *Brucella* genus, as shown by the fact that the description of the new *Brucella* species was given only by citing the original *Ochrobactrum* species publication, with no attempt to justify or explain the adequacy for the genus amendment in the current *Bergey's Manual of Systematics of Archaea and Bacteria* (5). Obviously, no *Ochrobactrum* strain is represented to any extent by *Brucella melitensis*, the type species that exemplifies the genus in this authoritative taxonomy manual (5).

The differences are even more evident for clinicians and workers in infectious diseases. While some free-living *Ochrobactrum* strains occasionally display opportunistic pathogen behavior in medically compromised patients, the brucellae do not multiply in the open environment; they are highly contagious intracellular pathogens endowed with an array of peculiar virulence adaptations, causing a long-lasting syndrome known as brucellosis (3, 6–8). In contrast, the few opportunistic *Ochrobactrum* strains are extracellular, inducing inflammatory disorders like those caused by other opportunistic bacteria, and lack true virulence factor genes (9–11). Thus, the diagnosis, anamnesis, prevention, epidemiology, and treatment of such infections depart from those of brucellosis. Moreover, *Ochrobactrum* species show broad antibiotic resistance encoded in the genome and plasmids. In contrast, *Brucella* organisms rarely develop antibiotic resistance, because of their lifestyle, lack of plasmids, and absence of contemporary recombination (Table 2). There are excellent serological tests for diagnosis of the most prevalent *Brucella* infections, while no serological tools are currently available for *Ochrobactrum* infections. Similarly, brucellosis in domestic ruminants can be controlled with vaccines, but such vaccines are not available or recommended to prevent *Ochrobactrum* infections. The list goes on (3). Illustrative of the unnecessary and serious confusion created, She et al. (12), on behalf of the ASM Clinical and Public Health Microbiology Committee, Laboratory Practices Subcommittee, recently elaborated a list of all known (thus far) *Ochrobactrum* "*Brucella* species," warning about the problems in the identification of the "true" brucellae when using some matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) approaches, nucleic acid detection methods, and automated phenotypic method databases. Examination of the simple tests provided to distinguish these obviously different bacteria also illustrates the uncertain basis of this merger.

Lumping these two bacterial groups into the same genus is unreasonable and unsafe, affecting the mainstream of science and creating risks for patients, veterinarians, medical doctors, laboratory workers, health authorities, and legislators who deal with brucellosis, which are particularly grave in low- and middle-income countries. *Brucella* and brucellosis have specific meanings depicted in textbooks, databases, and technical manuals regardless of the *Brucella* species since they produce the same syndrome, differing in virulence and host preference (6–8). Similarly, the widely different characteristics of opportunistic *Ochrobactrum* infections have been established (9–11).

It is difficult to know why the new taxonomic tag has appeared rapidly in influential databases, bacteriological collections, and online sources, including Wikipedia. Indeed, the proposed genus is in the List of Prokaryotic Names with Standing in Nomenclature (<https://psn.dsmz.de/genus/brucella>), and its easy accessibility could explain this fast spread. However, microbiologists (and institutions and databases) not familiar with the intricacies of the International Code of Nomenclature of Prokaryotes (13) are probably not aware that such a list is just a record of validly published names, i.e., those that appear in peer-reviewed journals and are then periodically listed in the *International Journal of Systematic and Evolutionary Microbiology* (in this case, in reference 14). Therefore, the names in the list are not official names endorsed by the International Committee on Systematics of Prokaryotes but a taxonomic "opinion" (*stricto sensu*) and not a scientific truth. In specific cases, these validly published names lack the support of working groups of experts in a bacterial group; significantly, the merging of *Ochrobactrum* and *Brucella* was launched without the input of brucellosis or *Ochrobactrum* specialists. What is probably not evident is that former names like *Ochrobactrum* remain validly published when an updated list with a new proposal appears, so that their preferential use is a choice open to acceptance by the interested parties.

Since the Swedish naturalist Linnaeus pioneered taxonomic work, taxonomy has provided names for living organisms, while phylogeny explores evolutionary histories. However, constructing phylogenies is one thing and formulating sets of codes for recovering information from a taxonomic scheme is quite another. Accordingly, taxonomy should be exercised as a responsible consensual understanding among the experts and parties interested in a bacterial group, especially when dealing with dangerous pathogens, and not routinely derived from quantitative phylogenetic information.

Names are not neutral, because they enclose information. As illustrated in Shakespeare's plot when Juliet Capulet asks Romeo Montague to disown his family name: "It's only your name which is my enemy. You are who you are, even if you weren't a Montague. What is a Montague? It's not a hand, nor a foot, nor an arm, nor a face, nor any other concrete part of the body. Oh, be some other name! What's in a name?" (15) And yet, because of their names, both lovers died in a cruel plot. This drama is not the story of star-crossed lovers but a tragedy of names shaping the destiny of two characters whose appellations represent an ancient quarrel impossible of reconciliation. Similarly, taxonomic names may have serious consequences if not adjusted to the realm of facts in microbiology, as in other fields (16). Therefore, taxonomy should be a system from which meaningful information is retrievable, not a perplexing arrangement of names disconnected from reality. What valuable information can be retrieved from names of soil bacteria such as "*Brucella ciceri*" (*Ochrobactrum ciceri*) or "*Brucella anthropi*" (*Ochrobactrum anthropi*)? Are chickpeas carrying "*B. ciceri*" risky for transmitting brucellosis, and should they be treated as vectors of pathogenic risk group 3 agents? Is environmental "*B. anthropi*" a pathogen with a preference for humans, as *Brucella ceti* is for dolphins and *Brucella canis* is for dogs? The most salient issue is how to deal with confusion without adding to it.

These issues are becoming increasingly relevant in clinical microbiology. Not surprisingly, the *Ochrobactrum-Brucella* case is not unique; similar unilateral rearrangements of nomenclature affecting other pathogens have followed and preceded. As expected, some have warned that similarly confusing new nomenclatures should be ignored (17, 18). Similarly, we advise using the *Ochrobactrum* and *Brucella* nomenclature, which, as stressed above, remains valid. The stewards of information, such as bacterial collections, genomic databases, encyclopedias, journals, reviewers, editorial boards, and scientists, must take into account these considerations in the process of reviewing, writing, and accepting unvindicated nomenclature proposals, acknowledging that *Ochrobactrum* is not *Brucella* and chickpeas are not cows.

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