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Whither Extensive Genomic-Based Microbial Taxonomic Revision?

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Whither Extensive Genomic-Based Microbial Taxonomic Revision?

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In the study published by Potter et al. in this issue of *Clinical Chemistry* (1), 103 genomes under the auspices of either Gardnerella vaginalis or Gardnerella spp. contributed to the National Center for Biotechnology Information database were accessed with the goal of further elucidating subspecies or new species designations (termed genomospecies in the context of in silico analysis). This genus is one of several that can contribute to the dynamic disease entity of bacterial vaginosis. A Canadian group has recently identified differentiating genetic patterns between bacterial vaginosis-, aerobic vaginitis-, and Lactobacillus spp.-dominated vaginal microbiomes (2). Intricate genetic characterization of novel taxa within the Gardnerella genus may identify target organisms for subsequent studies of pathogenesis.

In lieu of the traditional DNA-DNA hybridization standard, Potter et al. used 4 modalities of in silico analysis for subsequent genetic characterization of whole genome assemblies. These included 2 average nucleotide identity

(ANI)[3] platforms (3), tetranucleotide frequency (4), and average amino acid identity (AAI) (5). The authors used a conservative criterion for assignment of genomospecies (i.e., concordant results derived from ≥2 modalities of characterization). Classifications were supported by ancillary core genome, accessory genome, and metatranscriptome analyses. Using this algorithm, the authors report 9 Gardnerella genomospecies—verifying the taxonomic designation of Gardnerella piotii sp. nov. but suggesting potential conflicts in the taxonomic status of Gardnerella leopoldii sp. nov. and Gardnerella swidsinskii sp. nov. (6).

Past literature has espoused prokaryotic species-level ANI cutoffs of 94% to 96% (3). In a recent publication, Ciufo et al. (7) evaluated this threshold by iclinchem1346.xmldentifying 335 taxonomic designations for which at least 10 GenBank assemblies were available with ANI alignments above 10% coverage. These assemblies were compared with the submitted type taxon assembly and labeled as concordant (taxonomic agreement) or discordant (taxonomic disagreement). The average ANI for concordant comparisons was 97.1%, whereas the average for discordant comparisons was 86.3%. At a hypothetical threshold of 94%, 40 concordant pairs were observed with ANI values below that threshold (interpreted as false-negative matches), whereas 16 discordant taxa matches were observed with ANI values exceeding that threshold (interpreted as a failure to confirm the correct species). In contrast, establishing a threshold of 96% resulted in 77 concordant pairs with an ANI value below the threshold, with only 9 discordant taxa matches being observed with ANI exceeding that threshold.

Ciufo et al. ultimately espouse this 96% ANI cutoff value over 90% coverage of the genome of interest. These researchers estimate that the identities of two-thirds of genome assemblies residing in GenBank can be confirmed using ANI mclinchem1348.xmlethods compared with a type assembly. Approximately 4% are misidentified, and the remaining 30% cannot be evaluated because of a paucity of relevant type strain assemblies. At the same time, cutoff values have the potential to vary by taxon. Clearly defined relationships between members of a genus may result in high ANI cutoff values, such as those (approximating 98.8%) observed for Mycobacterium tuberculosis, Mycobacterium bovis, and Mycobacterium africanum. In addition, ANI cutoffs of 99.99% have been established for Streptococcus almquistii, Streptococcus avellaneus, and Streptococcus gibsonii. In contrast, less-defined intraspecies relationships may result in lower ANI cutoff values (such as 88.50% for Stenotrophomonas maltophilia and 93.50% for Lactobacillus gasseri) (7).

Tetranucleotide frequency analysis has contributed to variability determination of long DNA sequences in microbial genomes for >20 years. Noble et al. (4) reported a nonlinear relationship between variancesclinchem1350.xml of tetranucleotide frequencies and GC content upon comparison of 9 completely sequenced bacterial genomes. Greatest variances occurred in DNA sequences with low GC content. Richter and Rosselló-Móra (3) commented that tetranucleotide frequency values can assist in the classification of a group of strains into the same species and that an application of this modality may best lie in screening results of large data sets before more-discriminating ANI calculation. The data from Potter et al. support this notion, as tetranucleotide analysis revealed 9 genomospecies of Gardnerella spp. from the 103 G. vaginalis and Gardnerella spp. genomic deposits, with 12 and 14 in silico Gardnerella genomospecies designations derived from the 2 ANI modalities.

Konstantinidis and Tiedje (5) advocated AAI as an initial step toward a genome-based taxonomy because of its simplistic measure of relatedness for all prokaryotic taxa. These authors reported improved resoluticlinchem1357.xmlon of this technique over that of 16S rRNA characterization between closely related species. The authors also noted that the historic 70% DNA-DNA hybridization threshold for species delineation corresponded to 95% to 96% AAI. In silico characterization of Gardnerella spp. deposits by Potter et al. revealed 8 genomospecies via AAI. Similar to tetranucleotide frequency analysis, genomospecies differentiation of the type strains G. leopoldii and G. swidsinkii could not be accomplished by AAI (as it was via 1 format of ANI). Furthermore, both AAI and tetranucleotide frequency analysis failed to differentiate G. piotii from what the 2 ANI templates identified as a distinct genomospecies 4.

Within the past 5 years, attempts have been made on a regular basis to summarize important changes to the taxonomy of microbial agents derived from human clinical specimens (8, 9clinchem1361.xml). Such amendments can have a direct impact on how the clinical microbiology laboratory supports the clinical treatment of patients. One crucial facet of clinical microbiology practice (which can even fall under the purview of national laboratory accreditation standards) is the selection of appropriate antimicrobial agents to test against clinically significant isolates. The following example relates to the Gram-negative bacillus with previous designation of Actinobacillus actinomycetemcomitans. A 1985 taxonomic revision (10), resulting in organism classification within the genus Haemophilus, allowed for Clinical and Laboratory Standards Institute (CLSI)-sanctioned antimicrobial susceptibility testing of isolates using either disk diffusion or broth microdilution methods. Subsequent reclassification of this organism into the genus Aggregatibacter (11) resulted in its transition to CLSI susceptibility testing guidelines specific to HACEK group organisms (Aggregatibacter spp., Cardiobacterium spp., Eikenella corrodens, and Kingella spp.) (12). Within this CLSI M45 guideline, parameters do not exist for disk diffusion susceptibility testing. Furthermore, broth microdilution guidelines now require utilization of a more-nutritive cation-adjusted Mueller–Hinton broth supplemented with lysed horse blood. Moreover, the number of reportable fluoroquinolone and cephem agents is reduced to 2 apiece (from 9 and 15, respectively, in Haemophilus spp. guidelines) with introduction of penicillin and carbapenem interpretive criteria (12).

Although Potter et al. and others have embraced the prospect of genomic-based taxonomy in the context of microbial pathogenesis investigation, routine clinical microbiology practice has engaged these revisions, on occasion, as sources of controverclinchem1361a.xmsy, confusion, and even consternation. Phylogenetic examination of the Clostridium genus, particularly in the past 5 years, has uncovered significant diversity to the point that several members no longer demonstrate sufficient homology to the type species Clostridium butyricum. The genus/species designation “Peptoclostridium difficile” was originally proposed to reflect such differences in the former Clostridium difficile (9). Recognizing that this proposed revision would change the monikers of C. difficile or “C diff” already ingrained in commercial products, clinical laboratories, and laboratory information systems and to avoid some controversy among healthcare professionals, Lawson et al. (13) proposed a compromise with the new designation of Clostridioides difficile comb. nov., thereby retaining the commonly used abbreviation of C. difficile.

In 2017, the taxonomic designation of Enterobacter aerogenes was transferred to Klebsiella aerogenes comb. nov. (14). The clinical utility of this taxonomic revision remains to be seen, particularly with respclinchem1363.xmlect to salient differences in predicted antimicrobial susceptibility profiles of the Klebsiella and Enterobacter genera in general (particularly relevant to first-generation cephems, cephamycins, and β-lactam/β-lactamase inhibitor combinations such as ampicillin-sulbactam) and the confusion that this may present to clinicians. Adeolu and Gupta (15) proposed a reclassification of Borrelia spp. spirochetes in 2014. Agents responsible for Lyme borreliosis (formerly residing in the Borrelia burgdorferi sensu lato complex) were transferred to Borreliella gen. nov., while spirochetal agents largely responsible for relapsing fever retained the Borrelia genus designation (type species Borrelia anserina). Among agents now found within Borreliella gen. nov. include B. burgdorferi comb. nov. (type species of Borreliella), B. afzelii comb. nov., B. garinii comb. nov., and B. japonica comb. nov. In response to this, a consortium of 27 researchers (16) authored a paper in 2017 that strongly opposed this division of Borrelia spp. spirochetes.

Within the genre of folklore, cautionary tales were spun to warn audiences of a potential danger. Three essential components contributed to these monologues: declaration of a condition or scenario that was thought to be dangerous or taboo; subsequclinchem1375.xmlent narrative describing how someone disregarded the warning and engaged in the forbidden act; and the often unpleasant result of disregarding the taboo warning (which was often presented in gruesome detail). In the context of clinical medicine, clinicians encounter vast amounts of

data and informatics daily. How we as scientists (and clinicians) disseminate and even personally communicate these data in a clinically relevant fashion could have a tremendous impact on the care of our patients. Genomic-based microbial taxonomy possesses incredible potential to further affect the worlds of pathogenesis, diagnostics, therapeutics, and epidemiology in the years to come.

But just because we can, should we? At times, clinical microbiologists and infectious diseases specialists need to exhibit patience and recognize the value of a number of these taxonomic revisions—especially when such data may contribute toclinchem1388.xml a better understanding of disease. Variability in disease presentations previously attributed to host factors may now be associated with or linked to specific microbes. The publication by Potter et al. provides a compelling exemplification of this in the context of bacterial vaginosis pathogenesis. Perhaps the mitigating antidote to the potential "cautionary tale" of evolving genomic-based microbial taxonomy may lie in appropriate clinical perspective, appropriate clinical application, and proper communication of such advances in the field to our clinical partners.

Nonstandard abbreviations

- **ANI** average nucleotide identity
- **AAI** average amino acid identity
- **CLSI** Clinical and Laboratory Standards Institute.

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