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Update on Sexually Transmitted Infection Diagnostics

There were several key studies published in 2019 and a new FDA-cleared test to be aware of in the area of sexually transmitted infection diagnostics. **Mycoplasma genitalium**, a small bacterium (both geometrically and genomically), is a relatively frequent cause of urethritis or cervicitis; however, testing options have been quite limited until recently. Culture for **M. genitalium** is complicated because it is an obligate intracellular pathogen and, as such, requires host cell culture assays and replicates slowly. While non-FDA-cleared molecular assays have existed for some time [1], validating a molecular laboratory-developed test for the organism is fairly involved, particularly in the acquisition of appropriate numbers and types of specimens for validation. Hologic, Inc. (San Diego, CA), received FDA clearance for their transcription-mediated-amplification (TMA)-based Aptima **M. genitalium** assay this year. Given the lack of suitable comparator methods, it was noteworthy that they utilized a reference standard of 3 alternative TMA assays also targeting **M. genitalium**; the assay performed very well relative to the alternative assays [2]. In another study evaluating this Aptima assay, sensitivity was very good for vaginal or urethral swabs (98.9% and 98.2%, respectively) but markedly less sensitive for urine specimens (77% for female; 90.9% for male) [3]. Although this new test is available, it is not clear at this time which patients (or laboratories) would benefit from testing for **M. genitalium**. The organism demonstrates considerable resistance to frontline therapeutic agents (macrolides and fluoroquinolones), so molecular detection without phenotypic or genotypic susceptibility testing may have somewhat limited value. Future studies to (i) evaluate the cost-effectiveness of **M. genitalium** molecular testing compared to clinical diagnosis and empiric therapy and (ii) determine the best patient populations to target for testing would be helpful.

There was also an update on the HIV testing front, with publication of the RV254/South East Asia Research Collaboration in HIV 010 (RV254/SEARCH010) study and RV217 study findings by Manak et al. [4]. To date, this study has offered the clearest demonstration of the impact of antiretroviral therapy (ART) on diagnostic test results. Virologic suppression mediated by ART during acute infection significantly impaired seroconversion. Currently recommended and essentially universally implemented HIV testing algorithms work very well for patients not on ART. However, many study patients were taking pre-exposure prophylaxis (PrEP) ART. Based on the study’s findings, there is concern that patients on PrEP or with indirect access to ART who are poorly compliant or intermittently taking ART
will become infected and evade traditional diagnostic measures. Although these concerns probably do not justify a change in routine HIV testing algorithms, it may be reasonable to perform a nucleic acid amplification test (NAAT) despite a negative serologic screening test result in select patients.

Genotype Assays for Predicting Resistance Phenotype

There has been considerable recent progress in developing molecular tests to evaluate resistance mechanisms for clinically important organisms that are difficult (or slow) to culture and for which resistance to frontline antibiotics is a serious concern. As previously discussed, *M. genitalium* is difficult to culture and exhibits considerable resistance to fluoroquinolones and macrolides. A previously published report demonstrated excellent results for a multiplex PCR assay designed to detect *M. genitalium*, as well as mutations in *M. genitalium* 23S rRNA associated with macrolide resistance [5]. Fernandez-Huerta et al. [6] described an assay for simultaneous detection of *M. genitalium* and mutations to subunit A of topoisomerase IV (ParC) that lead to fluoroquinolone resistance. In both studies, multiplex PCR assays were compared to Sanger sequencing. Macrolide resistance was predicted in 63% of *M. genitalium* clinical isolates [5]; fluoroquinolone resistance was predicted in 8.8% (Spanish cohort) to 23.4% (Australian cohort) of *M. genitalium* clinical isolates [6]. A similar approach was taken to detect macrolide resistance in *Mycoplasma pneumoniae* in a national cohort [7], wherein investigators found 7.5% of isolates were predicted to be resistant to macrolides (the results also compared favorably to those of phenotypic susceptibility testing).

I have been consulted several times regarding susceptibility testing for *Helicobacter pylori* due to failed therapy. Although several reference laboratories provide culture and phenotypic antibiotic susceptibility testing (AST) assays, successful culture of *H. pylori* is often challenging due to storage and shipping requirements prior to culture setup at the reference laboratory. Nezami et al. [8] described a retrospective evaluation of next-generation sequencing for detection of mutations in *H. pylori* 16S rRNA, 23S rRNA, and gyrA from remnant formalin-fixed paraffin-embedded gastric biopsy specimens with correlation to the outcome of therapy that included clarithromycin [8]. *H. pylori* was successfully detected in 126/133 histologically positive specimens, of which 63 (50%) harbored at least one well-characterized point mutation, with an additional 29 specimens having mutations for multiple targets. Treatment outcomes were available for 58 patients, among whom 0/15 patients with no mutations failed therapy, 5/27 patients with one detected mutation failed therapy, and 11/16 patients with multiple mutations failed therapy. It would be great if such an assay proved to have clinical utility in a prospective study and became available at a reference laboratory.

Susceptibility testing for *Mycoplasma tuberculosis* complex (MTBC) is a very slow process but is critical for selecting drug regimens. Of particular importance is pyrazinamide (PZA) AST. There is a considerable body of literature on the impact of an MTBC gene with pyrazinamidase/nicotinamidase activity (*pncA*) on PZA resistance. Sequencing *pncA* to evaluate for mutations demonstrated very good sensitivity for detecting PZA resistance in a retrospective cohort of isolates with defined phenotypic AST profiles, as well as in a small prospective evaluation [9]. Of note, sequencing results were all available within 4 days during the prospective study compared to 35 days for phenotypic testing of the same isolates performed by the same laboratory. (Further discussion of this topic, i.e., genotypic assays for predicting an antibiotic resistance phenotype, is available in two excellent reviews published in 2019 [10,11]).

Phenotypic Susceptibility Testing

Phenotypic susceptibility testing for *mecA* in coagulase-negative staphylococci (CONS) has been a bit of a moving target. As noted in the Clinical Laboratory Standards Institute (CLSI) M100 (29th edition), using recommended methods and breakpoints still has limitations, especially for non-*Staphylococcus epidermidis* species. *Staphylococcus lugdunensis* was noted years ago to behave more like *Staphylococcus aureus* than other CONS species and, as such, was grouped with *S. aureus* for recommended methods and breakpoints. *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*, more common in veterinary practice, are not amenable to testing with cefoxitin (disk or MIC) but do share oxacillin MIC breakpoints with CONS (other than *S. lugdunensis*). CONS other than the species mentioned above have been listed as testable by oxacillin MIC or cefoxitin disc, but with a comment that oxacillin MICs between 0.5 and 2 μg/ml (resistant range) may overcall resistance for non-*S. epidermidis* species; such isolates should then be tested by a *mecA/PBP2a* detection method. A study published in 2019 evaluated 100 isolates of *S. epidermidis* (48 with and 52 without *mecA*) using both MIC and disc diffusion methods and both oxacillin and cefoxitin. The findings demonstrated that the best performance (100% categorical agreement) was actually achieved by oxacillin disc diffusion (not previously recommended for CONS) but using *S. pseudintermedius/schleiferi* breakpoints [12]. The study also noted excellent performance for a PBP2a assay (SA culture colony test; Abbott Diagnostics, Abbott Park, IL). In light of these findings, it may be safest to just use this type of assay for any CONS isolate isolated from a serious infection that is near the recommended breakpoints for the respective *Staphylococcus* species until this is sorted out. Additional modifications for other CONS are probably coming soon [13].

Clinical utilization of colistin had fallen out of favor due to toxicity concerns but came back around in the last decade as an option for extensively drug-resistant Gram-negative rods [14]. Susceptibility testing for colistin has been an issue for most laboratories, however, as there has been only one CLSI-endorsed testing method, broth microdilution (BMD), and most laboratories do not perform BMD. Colistin is not included on FDA-cleared panels (there are no FDA breakpoints for colistin), and disc diffusion and gradient diffusion do not work well for colistin. So, do you just send an isolate to a reference laboratory for colistin? Of note, two 2019 publications highlighted a new method that appears to work well for determining colistin resistance in *Enterobacteriaceae, Pseudomonas aeruginosa*, and *Acinetobacter* spp. [15,16]. The colistin broth disc elution method involves adding 0, 1, 2, or 4 colistin discs (10 μg
each) to 10-ml tubes of cation-adjusted Mueller-Hinton broth, followed by a 0.5-McFarland standard suspension of the test organism in saline, and incubation at 35°C for 16 to 20 h before reading the tubes visually for growth to determine the MIC (the tubes have 0, 1, 2, or 4 μg/ml colistin, key concentrations for interpretation of susceptibility results). Any clinical microbiology laboratory that performs susceptibility testing should be able to perform this test, delivering reliable results without the delay and expense of sending isolates to a reference laboratory. It may also work well to perform colistin AST using matrix-assisted laser desorption ionization—time of flight (MALDI-TOF) [17], but this will need further evaluation.

There were several updates in carbapenemase detection and characterization published in 2019. Sfeir et al. [18] described the EDTA-modified carbapenem inactivation method, which can be used to determine whether carbapenemase activity is due to a metallo-β-lactamase (MBL). MBLs require zinc for activity, and zinc is chelated by EDTA; this distinction is important due to differences in therapeutic options [19]. Several studies evaluated using modified carbapenem (or other β-lactam) inactivation assays directly from blood culture bottles with good results [20–23].

Although not new in 2019, the Accelerate PhenoTest BC (Pheno assay; Accelerate Diagnostics Inc., Tucson, AZ) represents one of the major technological advances in the field in recent years, providing phenotypic AST results within 8 h from positive blood cultures. A study published in 2019 compared the Pheno assay to direct inoculation in the Vitek automated AST (Vitek 2 system; bioMérieux, Durham, NC) [24]. Compared to reference broth microdilution AST results for 86 positive blood cultures, the Pheno system had categorical agreement for Gram-negative and Gram-positive organisms of 92.7% and 99%, respectively; very major (VME), major, and minor error rates were 3.6% (6/166), 2.2% (9/416), and 3.8% (23/600), respectively. For direct Vitek, categorical agreement was 91.7% for Gram-negative organisms and 99% for Gram-positive isolates; very major error, major error, and minor error rates were 2.4% (4/169), 1.0% (4/416), and 5.8% (35/603), respectively. Five of the 6 VMEs for the Pheno assay occurred with Klebsiella isolates, and half (2/4) of the VMEs for direct Vitek were for colistin (the study was performed in Europe using EUCAST breakpoints; colistin is not included on commercial panels in the United States). The major advantage of a technology like the Pheno assay is the major decrease in time to reporting AST results. Many studies have compared before and after implementation of Pheno assays and demonstrated massive reductions for initial phenotypic AST reporting compared to conventional methods. Unfortunately, the study cited above with direct Vitek AST did not perform an evaluation of time to availability of AST results. Further studies will hopefully determine the impact of providing faster AST results on patient outcome and cost-effectiveness for various approaches to rapid AST from blood cultures.

In 2019, a major change was made for fluoroquinolone breakpoints by CLSI; changes had also been made for fluoroquinolones by EUCAST in 2017, but the CLSI changes were somewhat more limited in scope. The breakpoint changes were made in response to evidence that the previous breakpoints did not capture low-level resistance in isolates harboring known resistance mechanisms. The breakpoints for Enterobacteriaceae and P. aeruginosa were lowered significantly; the changes and the rational behind them were discussed in more detail in a recent review [25].

There have been continued discussions about the differences in the CLSI and EUCAST recommendations for the AST interpretive criteria and result-reporting structure, as well as the underlying differences in strategies to establish such recommendations. A point-counterpoint on the topic with authors from both sides of the Atlantic Ocean was published in 2019 [26]. There has also been discussion about the topic on the ClinMicroNet laboratory directors’ listserv. While many clinical microbiologists who have engaged in the discussions prefer that the organizations merge or cooperate more extensively, I will put out my opinion that I hope they stay very independent. I think it is valuable to have two separate organizations of experts approaching the same critically important topics with different perspectives, and I regularly refer to each.

**Resistance Threats and New Drugs**

In 2019 the Centers for Disease Control and Prevention (CDC) released a report entitled “Antibiotic Resistance Threats in the United States”; they had previously produced the same type of report in 2013. The 2019 CDC report provided an expansion and update on the former report. The 2019 report included several pointed statements, such as “there is no safe place from antibiotic resistance” and “stop referring to a coming post-antibiotic era—it’s already here.” While there certainly is cause for concern regarding antibiotic resistance, I think such statements are alarmist, and the latter is inaccurate. The report defines 18 specific organism resistance threats and provides estimates of whether rates of the resistance threat have increased or decreased since the prior report in 2013. Allowing for updates to the 2013 data in some areas, the CDC estimated that rates for 6 of the 18 specific organism resistance threats had decreased (including vancomycin-resistant enterococci, multidrug-resistant P. aeruginosa, carbapenem-resistant Acinetobacter baumannii, methicillin-resistant S. aureus [MRSA], Clostridoides difficile, and resistant Candida spp.) and that 5 of 18 had increased (including extended-spectrum beta-lactamase–producing Enterobacteriaceae, resistant Neisseria gonorrhoeae, resistant non-typhoidal Salmonella, Candida auris, and erythromycin-resistant group A Streptococcus). The threats that have decreased had more clinical impact than the threats that increased in terms of the mortality and morbidity estimates provided in the report. It seems likely that we should credit the dedicated work of antimicrobial stewardship groups and infection preventionists for helping to restrain resistance development and spread to the extent possible, and hopefully these types of resources can be extended globally. Additionally, 2 functionally novel antibiotics were FDA approved in 2019: cefiderocol (for complicated urinary tract infection [cUTI]) and lefamulin (for community-acquired pneumonia) [27,28]. Another β-lactam/β-lactamase inhibitor combination drug was also FDA approved in 2019 (imipenem-relebactam for cUTI
and complicated intrabdominal infections), as well as a promising new drug for the treatment of extensively drug-resistant *M. tuberculosis* (pretomanid for use in combination with linezolid and bedaquiline). Some of the “forgotten antibiotics” [29] may also make a comeback, with intravenous fosfomycin submitted for FDA evaluation in 2019. With new drugs come requests for testing before you are ready for them—James Kirby’s laboratory published an interesting discussion on the approach to testing new drugs in 2019 [30].

**Clostridioides difficile Laboratory Testing**

*C. difficile* testing has seen some major changes in the last few years. In 2018, the National Healthcare Safety Network (NHSN) changed the rules on reporting cases of hospital-acquired *C. difficile*, which I am sure your infection control group told you about if you did not hear it somewhere else first [31]. This change will have an impact on *C. difficile* testing method selection and hospital-acquired infection (HAI) statistics and is likely to increase health care costs without any obvious benefit for patients. Several studies have evaluated ultrasensitive *C. difficile* toxin (protein) detection methods, one of which was briefly marketed in the U.S. in 2018/2019 (Singulex Clarity, Inc., Alameda, CA). A 2019 study comparing this particular ultrasensitive *C. difficile* toxin A/B assay to NAATs and cell cytotoxicity neutralization assay demonstrated improved specificity relative to NAAT alone [32]; improvements in sensitivity relative to other toxin assays on the market had been demonstrated previously [33]. While such a testing method has some clear virtues, there are also some drawbacks. The Singulex system required a separate and expensive piece of equipment for a single test. Another 2019 study evaluating a different ultrasensitive *C. difficile* toxin assay (Simoa; Quanterix Corp., Billerica, MA) demonstrated no statistically significant difference in toxin concentrations between NAAT™ *C. difficile* infection patients and NAAT™ carriers without diarrhea [34]. Additionally, a lower limit of detection for a toxin-based assay would effectively lead to markedly worse HAI numbers, as NHSN adjusts expected case numbers based on NAAT/toxin-based testing (expecting fewer cases for toxin-based testing). Singulex also ran into a major regulatory issue: their *C. difficile* Clarity assay was registered with the FDA, but they did not apply for or receive 510K approval, apparently believing they did not need it. They were contacted by the FDA in 2019 and told to stop marketing the assay. Whatever the considerations were, either regulatory hurdles or a lack of interest in the Singulex assay/system, the company went out of business in June 2019.

**Metagenomic Sequencing Goes Mainstream**

Metagenomic sequencing (mNGS) directly from clinical specimens is now readily available through reference laboratories, and there were several key papers on the topic in 2019. A large study involving the University of California—San Francisco clinical microbiology laboratory and several other clinical centers prospectively evaluated the utility of mNGS on cerebrospinal fluid (CSF) to aid in the diagnosis of infectious meningitis/encephalitis [35]. Among 204 patients, 58 were diagnosed with a central nervous system infection, which included 45 diagnosed by routine testing methods (19 of which were also identified by mNGS), and an additional 13 infections were detected by mNGS alone. The results were communicated in real time to patient providers. Of note, 8 of the 13 cases identified by mNGS alone were considered to be clinically significant. Some of the additional identifications could possibly have been detected (but were not) by routine tests that were ordered (including *Streptococcus agalactiae*, *Klebsiella aerogenes*, *Streptococcus mitis*, *Nocardia farcinica*, *Neisseria spp.*, *Enterococcus faecalis*, and *Candida tropicalis*) or could have been ordered (2 enterovirus cases). Others were viral infections not in the provider's differential (St. Louis encephalitis virus and hepatitis E virus), of unclear significance (MW polymavirus), or not related to an acute infectious process (Epstein-Barr virus [EBV] in a patient with EBV-positive lymphoma-associated encephalitis). Among 26 infectious cases for which mNGS was negative, 11 were diagnosed by serologic testing only, 7 had positive routine testing results from specimens other than CSF, and 8 were positive by routine testing performed on CSF. Establishing the threshold for calling a positive result for mNGS is critically important: all 6 cases of bacterial or fungal infection detected by routine culture or PCR from CSF that were “missed” by mNGS (including *M. tuberculosis*) actually had reads corresponding to the target in question detected, but below the established threshold. Even with a conservative threshold, mNGS had three positive calls (*S. aureus*, *S. agalactiae*, and *Pantoea* spp.) that were determined to be false positives (likely sample/environment contaminants) after discrepancy testing. An additional 19 viral agents were detected by mNGS that were felt not to be related to the clinical presentation. In summary, mNGS from CSF appears to have adjunctive value in addition to routine testing. Nevertheless, careful thought will be required for both patient selection and result interpretation (24 organism detections were considered either non-contributory or falsely positive compared to 32 organism detections that were the basis of or consistent with the final diagnosis).

A commercial laboratory also now offers mNGS on cell-free DNA in circulation with testing on direct patient blood specimens with a rapid turnaround-time [36]. Several studies used this service to evaluate dozens of patient specimens, with mixed results [37-39]. In two studies, nearly half of all positive results were for two or more organisms (range, 2 to 8) [39,40]. Clearly, mNGS has tremendous advantages in being able to detect nucleic acid from virtually any organism and has provided diagnoses in cases where routine methods failed or appropriate esoteric testing was not considered, but thoughtful studies are needed to determine when to employ the method. Current sample collection methods focus on sterilization of the collection site, but perhaps for mNGS, new guidance is necessary to prevent skin or environmental microbial nucleic acid specimen contamination, whether it is associated with viable organisms or not.

There have also been increasing efforts to promote mNGS testing for other diagnostic scenarios, such as orthopedic joint revision infections and even chronic UTI. It is not yet clear if
this will be beneficial for patients, including consideration of cost-effectiveness.

The above discussion describes reference laboratory work, but mNGS may also be coming to hospital and public health laboratories near you in the near future (if it has not already) [41]. Should it? Another great Journal of Clinical Microbiology point-counterpoint addressed this question in 2019 [42].

A Few More Random Studies

A few more quick hitters, ranging from elegantly practical to pushing boundaries, are provided with limited discussion below. You may be able to improve acid-fast staining for acid-fast bacilli using bulk containers (instead of rack-based methods) and an acetone rinse step; the study authors observed more bacilli per slide with the acetone rinse and had less background noise resulting in shorter reading times [43]. If you are looking to bring in MALDI-TOF for Mycobacteria (or to update your current approach), experts on this topic at the National Institutes of Health published a detailed protocol for one-step extraction that significantly reduces processing time with good identification results [44]. With the use of a specimen-type-specific database, monomicrobial UTIs may be able to be identified rapidly by MALDI-TOF [45]. CRISPR-Cas biology has been evaluated for both diagnostic and therapeutic applications [46].

Clinical Microbiology Education—Times Have Changed

Best practices, testing methods, and even organism names are sometimes moving targets in clinical microbiology, and it is important for the clinical microbiology professional to stay up to date. There are a variety of ways to keep up with the new (and learn some of the old, too), including reference books, attending conferences or watching webinars, and reading clinical microbiology- and infectious disease-oriented journals. In recent years, there have been increasing educational content availability and utilization on the Internet. The American Society for Microbiology has a clinical microbiology-oriented “Bugs and Drugs” blog where you can learn how MALDI-TOF or mNGS works, for example, or get updates on a variety of organisms or testing methods, and a new monthly virtual journal club where laboratory directors present and discuss recent research papers (including many of the ones cited above) via live video stream. If you have a few free minutes on your commute or lunch break, you can hit Twitter and see great educational microbiology cases from the laboratory (see @richdavisphd) or get up-to-the-minute news on what has been published in Journal of Clinical Microbiology (see @JClinMicro), including the cartoon section.

Summary

2019 was a big year in clinical microbiology that brought us new technologies, new approaches to old problems, new taxonomic changes, and a new coronavirus [47,48], among other things. While I have highlighted some of the studies and perspectives that stood out to me, many other important topics were not discussed. It is a distinct privilege for me to work in this field that is both exciting and dynamic, as well as practical and service oriented, and I look forward to another year being part of the great community of clinical microbiologists.

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