Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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IN THIS ISSUE

Clinical Microbiology in 2021: My Favorite Studies about Everything Except My Least Favorite Virus

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Abstract

Maybe 2021 wasn't so bad after all! It was like 2020, but with COVID vaccines and better access to plastic pipette tips, and clearly, 2022 is getting off to a bad start. Even better, 2021 had some exciting papers in clinical microbiology, which is what is highlighted in this 2021 year in review. None of them are about COVID-19. Of course, there were some interesting discoveries about SARS-CoV-2 and COVID-19, but this review is completely COVID-19 free (almost).

Parasitology

Let's start off with an interesting but troubling study in parasitology. Malaria testing is obviously important, and a testing solution should ideally be available in hospital laboratories 24/7. Preparation of thin and thick smears for Wright/Giemsa staining and microscopy is the preferred method, but staff competent to prepare and read such stains may not be available at many locations or on all shifts. Rapid antigen tests have relatively good sensitivity for *Plasmodium falciparum*, the primary agent causing malaria cases seen in patients seeking care in the United States and can be used for a rapid result while waiting for the microscopy review. Malaria antigen tests are based on detection of some combination of pan-*Plasmodium* protein aldolase and/or lactate dehydrogenase, and *P. falciparum*-specific histidine-rich protein 2 (HRP2) or HRP3. The only FDA-cleared malaria antigen assay in the U.S. is the Abbott BinaxNow test, which detects aldolase and HRP2 (for which there is some cross-reactivity with HRP3). An important report out this year demonstrates that a considerable quantity (about 10%) of *P. falciparum* strains circulating in Ethiopia are losing HRP2 and HRP3 due to chromosomal deletion, possibly under selective pressure related to the tests commonly used in the region to detect and treat cases [1]. This is important to consider for testing methods in the U.S., as well. The BinaxNow assay utilizes aldolase as a pan-*Plasmodium* target, but this has lower sensitivity than HRP-based detection, as demonstrated by the lower sensitivity of the test for non-*falciparum* species of *Plasmodium*. If HRP2 and/or HRP3 is lost, the BinaxNow test would likely have a higher limit of detection for *P. falciparum*, leading to reduced sensitivity in low-parasitemia cases and would also mischaracterize some *P. falciparum* infections as non-*falciparum Plasmodium* species.

Mycology

1,3-ß-D-Glucan and galactomannan

2021 was a great year for clinical mycology papers. Several research papers are highlighted below, including *Candida auris* culture methods, updates on fungal antigen tests used to diagnose invasive fungal infection, and several interesting reviews.

Given that culture-based methods have limited sensitivity for invasive fungal infection (IFI), there is considerable interest in, and utilization of, alternate testing modalities, including fungal

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biomarkers, such as 1,3-β-D-glucan (BDG) and galactomannan. Although the appeal of a test using non-invasive specimen types that can improve detection of IFI is obvious, there are concerns about the accuracy of these tests. For example, BDG testing for invasive candidiasis is estimated to be about 75 to 80% sensitive and 60 to 80% specific. Galactomannan testing for invasive aspergillosis using a 0.5 cutoff value is approximately 78% sensitive and 85% specific [2]. There is still a lot of room to debate their utility [3], but if you are running these tests in your laboratory, and especially if you are sending them to a reference laboratory, there was good news in 2021 regarding the possibility of attaining the same mediocre performance much faster!

Let's start with BDG. While living in Boston, I visited Cape Cod a few times, and one of its endearing features is that everything seems to move more slowly there, including BDG testing deveoped by the Associates of Cape Cod, Inc., the developers of the original Fungitell BDG assay. However, they have now made a STAT BDG assay using a simple reader device that can provide BDG results in less than an hour. White et al. evaluated 107 serum specimens across a spectrum of BDG concentrations as determined by the Fungitell assay and noted equivalent performance between the two tests [4]. Unlike many exciting new tests, the STAT BDG assay is already FDA cleared, so you can bring this test in house more easily if you are so inclined.

In addition, rapid galactomannan testing is not far behind. Jani et al. evaluated the sona *Aspergillus* galactomannan lateral-flow assay (GM LFA) in serum and bronchoalveolar lavage (BAL) fluid with comparison to the Platelia *Aspergillus* galactomannan enzyme immunoassay (GM EIA), but also clinical categorization as proven, probable, or possible invasive aspergillosis (IA) [5]. Of 448 serum specimens, 8 were positive by both GM EIA and GM LFA, 8 were positive by only GM LFA, and 432 were concordantly negative. Of 85 BAL specimens, 2 were positive by both GM EIA and GM LFA, 6 were positive by only GM LFA, and 77 were concordantly negative. So, does the LFA have better sensitivity or worse specificity? The study also categorized 28 patients (31 specimens) with either positive fungal biomarker or positive *Aspergillus* cultures based on clinical evaluation and other available laboratory data as proven IA, probable IA, possible IA, or no evidence of IA. Among these, the GM LFA was positive in all 4 patients with proven IA, 7/8 with probable IA, and 2/4 with possible IA, but also 8/12 with no evidence of IA. In contrast, the GM EIA was positive in only 1/4 patients with proven IA and none with possible IA. Based on this patient categorization, it seems the answer is that the GM LFA is both more sensitive and modestly less specific than the GM EIA in this patient population. The GM LFA was read both manually and with a manufacturer-provided reader device, and interestingly, of the 14 specimens positive by GM LFA but negative by GM EIA, 12 were positive by the automated reader but negative by manual reading.

Coccidioidomycosis

Coccidioidomycosis is another diagnostic challenge that has been complicated by the fact that no single test achieves high sensitivity. Kassis et al. published a study wherein multiple testing modalities (IgG and IgM antibodies by different methods and antigen in urine or serum) were evaluated on more than 100 patients with established *Coccidioides* infections. Greater than 90% sensitivity was achieved by stacking immunodiffusion IgG and IgM in combination with urine antigen and serum antigen (4 tests total) while maintaining >95% specificity [6]. *Coccidioides* was detected by histopathology review in 55% and by culture in 60% of the cases and by pathology or culture in 66% of the cases. Unfortunately, the authors did not evaluate the sensitivity and specificity of layering one or more antibody/antigen tests (which are expensive and are sent to a reference laboratory) with the pathology/culture results (since these are typically performed routinely).

Another study out of Arizona, a *Coccidioides* hotspot, demonstrated promising results for a single test that attained relatively high sensitivity for coccidioidomycosis. Grill et al. developed a quantitative inhibition-based ELISA for detection of CTS1 (coccidioidal chitinase 1) in serum [7]. Among patients with proven or probable *Coccidioides* infections, the sensitivity was 87% when a cutoff that yielded 97% specificity for control subjects living in Phoenix, AZ, was used. Pretty promising!

Candida auris

Candida auris is frequently multi-drug resistant and is increasingly reported in the U.S. either as a causative agent of infections or as a part of environmental surveillance efforts [8]. Although *C. auris* is readily identified by matrix-assisted laser desorption–time of flight mass spectrometry (MALDI-TOF MS) and molecular methods where available, biochemical methods struggle to identify *C. auris*, with possible misidentification as *C. haemulonii*, *C. duobushaemulonii*, *C. parapsilosis*, *C. sake*, or other less common yeasts. For screening cultures, it is preferable to have a selective and differential agar (such as a chromogenic agar) capable of accurately identifying *C. auris*. A study by de Jong et al. evaluated 3 chromogenic *Candida* agars and 2 newly developed chromogenic agars—CHROMagar *Candida* Plus (CCP) and HiCrome *C. auris* MDR selective agar (HAMA)—that were specifically formulated to aid in the identification of *C. auris* [9]. None of the original chromogenic *Candida* agar formulations (CandiSelect, CHROMagar Candida, and Chromatic Candida) provided a meaningful ability to distinguish *C. auris* from other, closely related *Candida* species. In contrast, the CCP colony morphology/color pattern for *C. auris* could be distinguished from all but *C. vulturna* and *C. pseudohaemulonii*. The HAMA medium formulation includes inhibitory substances intended to select for highly drug-resistant strains, and in this study, only *C. auris* isolates with high fluconazole MICs (>256; 3/9 isolates) were isolated, thus limiting utility. Another paper by Das et al. describes the development of a simple selective agar for *C. auris*, modifying a base of yeast extract-peptone-dextrose agar with 12.5% NaCl and 9 mM ferrous sulfate (SAM medium) for incubation at 42°C for 48 to 72 h. Of 133 *C. auris* isolates tested, 127 grew on this medium by 48 h, the remaining 6 isolates grew by 72 h, and none of the 446 non-*C. auris* yeast isolates from an extensive library of common clinical yeasts were able to grow within 72 h [10].

Molecular diagnostics

Your laboratory has probably sent out (or performed in house) broad-range fungal PCR with amplicon sequencing for the detection and/or identification of fungal pathogens in tissue. But does it actually help? Lieberman et al. evaluated data from 52 patients with suspected fungal sinusitis who had tissue submitted for culture and broad-range fungal PCR on the same day (54 specimen pairs). Of 49 specimen pairs categorized as proven, probable, or possible IFI, 27 were positive by culture (55%) and 33 were positive by broad-range PCR (67%). Of note, the turnaround time for PCR-based testing was shorter than for culture, though this would not be true for testing sent to a reference laboratory (testing for this study was performed in house). Specimens that were positive by direct stain were detected equally well by culture and PCR (18/19 for each), and PCR had the advantage in smear-negative cases. It is also worth noting that the majority of patients received anti-fungal drugs prior to testing, which may have impacted the sensitivity of culture to a greater degree than that of PCR. The authors concluded that the improved performance of PCR impacted clinical decision making in 16.7% of cases, which is pretty compelling [11]. In addition, although formalin fixation and paraffin embedding (FFPE) are known to degrade nucleic acid, in a larger data set (not matched pairs), a higher positivity rate for FFPE specimens (42.3% of 208 specimens) than fresh specimens (34.6% of 436 specimens) was found. This may reflect better specimen selection for FFPE, where blocks with detected organisms can be chosen versus blind testing on fresh specimens; regardless, this high level of detection from FFPE is surprising.

2021 Reviews of fungal diagnostics

Several helpful reviews on fungal testing topics were published in 2021. Lamoth et al. performed a systematic review of the performance of BDG serum testing in various clinical settings (excluding *Pneumocystis jirovecii* pneumonia (PJP)), finding that due to limitations of either low negative predictive value or low positive predictive value, routine BDG could not be recommended except to rule out IFI in solid-organ transplant patients and other immunosuppressed patients with low IFI risk or to start antifungal therapy with two consecutive positive BDG results in hematologic cancer patients at high risk for IFI or intensive care unit patients at high risk for invasive candidemia [12]. The Fungus Testing Laboratory and Molecular Diagnostics Laboratory at the University of Texas Health Science Center at San Antonio, TX, published a 1-year review of mucoralean molds identified by their national reference laboratory, including epidemiological data (11 genera and 25 species identified) and antimicrobial susceptibility testing (AST) for amphotericin B, isavuconazole, itraconazole, and posaconazole [13]. Nathan Wiederhold also published a primer for clinicians on antifungal susceptibility testing, which may be useful as educational material for your clinical trainees [14]. If you like big data sets, you might enjoy Table 1 of the study by Desnos-Ollivier et al. summarizing azole AST for over 9,000 yeast isolates representing 40 clinically relevant species [15].

Automation and Technology

Now, let's talk about computers and new technology. First off, there is little reason for any current or future technologist anywhere to think that automation or artificial intelligence is going to prevent them from working in a clinical microbiology laboratory—some efficiencies will be gained, but at a lower rate than the developing shortage of technologists. There are, and always will be jobs available for the medical technologist in microbiology.

But back to the computers. Wang et al. evaluated the ability of a convolutional neural network trained to evaluate Gram stain images to read vaginal microbiota smears and categorize their bacterial vaginosis Nugent score compared to technologists and obstetricians [16]. The gold standard "true" result for each image was determined by readings from two trained clinical microbiologists. If the clinical microbiologists disagreed on the Nugent score category (0 to 3, 4 to 6, or 7 to 10), a third reader (chief obstetrician) read the image, and if they agreed with one of the microbiologists, that result was accepted as true. There were some cases (number not provided) where none of the three agreed with each other, and there are only three categories! This type of assay is obviously subjective, but the computers performed pretty well and, depending on how they were tuned, could achieve up to 97% sensitivity, but with low specificity (69%). The best balance of the two was 89% sensitivity and 85% specificity. Individual technologists performed similarly to the high-sensitivity model; obstetricians were better for specificity.

There are two companies currently offering total laboratory automation (TLA) solutions for clinical microbiology laboratories, and if you reach out to them, they will both be happy to tell you how much money you will save if you purchase their automation system (based on your volumes and workflow). By "total" laboratory automation, what we are talking about is automating aerobic (not anaerobic) bacterial cultures from liquid specimens. This does not include anaerobic, fungal, or mycobacterial cultures, so it is not quite "total" automation. Culbreath et al. have published a nice comparison of what happened in four clinical microbiology laboratories before and after automation implementation [17]. In general, there were labor savings per specimen processed, which came in the form of processing more specimens with similar numbers of technologists. However, the calculations for cost savings in this study do not appear to account for the cost of the system, either capital expenditure up front or the ongoing cost of service, which can be substantial. Of interest, an additional finding was improved turnaround time for final result reporting following TLA implementation.

Cherkaoui et al. published an excellent study on utilizing a TLA system (Copan WASPLab) with the Colibri and Radian systems for setting up disk diffusion AST and Radian Expert System software to automate reading AST with comparison to an automated antimicrobial susceptibility testing system (VITEK 2) [45]. Categorical agreement between the two systems was accepted as the true result, while discordant isolates were evaluated by Sensititre broth microdilution or Etest gradient diffusion. Following resolution of

discrepancies, it was clear that the automated disk diffusion system performed quite well—even better than VITEK 2: among 718 isolates, the WASPLab/Radian disk system had only 2 very major errors (VME) and 18 major errors (ME) versus the VITEK 2, which had 45 VME and 9 ME. Overall categorical agreement was 98.6 to 99.4% for the various groups of organisms. Most of the VME for VITEK 2 were among Gram-negative bacilli, and some were *Pseudomonas* isolates with heteroresistant subpopulations, the kind of thing you might catch with disks better than with a black box broth system [18].

Mycobacteriology

Diagnosis of *Mycobacterium tuberculosis* in children is notoriously difficult. Kabir et al. studied the use of both standard Xpert MTB/Rif and the Xpert Ultra PCR assays compared to culture for both induced sputum and stool in children in Bangladesh [19]. There were 447 patients with paired sputum and stool specimens. For induced sputum, only 9/447 were culture positive, 12/447 were Xpert positive, and 28/447 were Xpert Ultra positive. For stool, 2/447 were culture positive, 11/447 were Xpert positive, and, amazingly, 60/447 were Xpert Ultra positive. Of the 28 positive sputum samples and 60 positive stool samples by Xpert Ultra, 11 and 48, respectively, were positive as "trace call," or low-level detection. The Ultra assay has established a lower limit of detection and improved sensitivity for respiratory specimens, but the dramatically higher rate of detection in this study was still surprising. One limitation to this study is that there was minimal evaluation to help determine if these were true positives or potentially false positive due to cross-reactivity with other DNA. Of note, the physicians involved with the study elected to treat the majority of the children who were positive at only the trace call level. It is unclear what to make of the additional detections in stool, but this study does highlight that it is *ultra*-frustrating that this *M. tuberculosis* assay with superior sensitivity is not available in the U.S. and is not expected to be anytime soon.

Rapidly growing mycobacterial species are increasingly a cause of skin, soft tissue, and respiratory infections, particularly *Mycobacterium abscessus* complex organisms. Macrolides are an important therapeutic option for *M. abscessus*, which may harbor inducible macrolide resistance through the *erm*(41) gene or constitutive resistance due to mutations in the peptidyltransferase domain of the 23S *rrl* gene. This can be variable, as in *M. abscessus* subsp. *abscessus*, with most having inducible resistance via functional *erm*(41) but with a significant minority that are susceptible. In contrast, *M. abscessus* subsp. *massiliense* is typically susceptible due to a truncated *erm*(41) gene, and *M. abscessus* subsp. *bolletii* typically has inducible resistance with a functional *erm*(41) gene [20]. Complicating matters, laboratory methods such as MALDI-TOF MS cannot differentiate *M. abscessus* complex subspecies, thus requiring sequencing-based identification and extended (14-day) AST to detect inducible resistance in *M. abscessus* subsp. *abscessus*. However, Marras et al. have demonstrated excellent performance for a 2-reaction probe-based PCR assay both to determine the subspecies of *M. abscessus* complex and to detect 23S *rrl* mutations and *erm*(41) truncation (*M. abscessus* subsp. *bolletti*) or point mutations (T28C) [21]. The assay performed well compared to whole-genome sequencing-based identification of the subspecies and modified genetic targets associated with resistance (100% correlation), although phenotypic susceptibility testing was performed in only a few isolates. Still, it is an interesting assay and a nice primer on molecular laboratory-developed test design.

Interferon-gamma release assays

Interferon-gamma release assays (IGRAs) test for memory T-cell responses to pathogen-specific antigens and can be used to determine whether a patient has a latent tuberculosis (TB) infection or to enumerate T-cell responses to cytomegalovirus, for example. Ward et al. noticed an increase in the rate of indeterminate QuantiFERON Gold Plus (a TB IGRA) results during the first few months of the COVID-19 pandemic in 2020 due to a poor response to lymphocyte activating mitogen in the assay control tube [22], which was associated with COVID-19 status in hospitalized patients. This was observed in our hospital, as well [23]. Ward et al. further evaluated cytokine responses in the blood of severely ill COVID-19 patients and found that 60% were unable to produce interferon gamma in response to phytohemagglutinin, even those with normal leukocyte counts. Reduced interferon gamma and increased interleukin 6 production suggest a TH2-skewed immune response in these patients. All right; COVID-19 will not be mentioned again.

You may have gotten a call at the laboratory before from a physician questioning a low-positive TB IGRA result that did not make sense based on the patient's clinical picture or exposure history. Wikell et al. reviewed results for Quantiferon Gold Plus TB IGRA testing on 58,539 patients in Sweden and evaluated the likelihood that patients with low-positive results would test negative on repeat testing versus how many such patients would go on to develop active TB infection during a 2-year follow-up period [24]. Among patients with borderline results (TB-nil of 0.20 to 0.99 IU/ml; the manufacturer cutoff for positive is 0.35 IU/ml) who had follow-up within a median of 36 days after the initial test, 480 of 1,254 were negative on the repeat testing, and none of the patients with repeat negative results developed active infection during the follow-up period. Two patients with borderline negative (0.2 to 0.34) and 2 patients with borderline positive (0.35 to 0.99) were positive (≥1.0) on repeat testing and subsequently developed active TB infection. The authors also performed an extensive evaluation of the contribution of the TB2 tube (new for the Plus assay), which can be summarized by saying it contributed very little to identifying latent tuberculosis infection (LTBI) cases.

Resistance Detection by Genotypic Methods

Marrero Rolon et al. published a study of the analytical and clinical performance of a PCR-based assay to detect *Helicobacter pylori* and predict resistance to clarithromycin [25]. Among 524 stool specimens, with comparison to an *H. pylori* stool antigen test, the PCR assay was 89% sensitive and 97% specific. Of the 113 PCR-positive specimens, 77 had a wild-type prediction (68%) and 36 (32%) had at least one single nucleotide polymorphism in the *H. pylori* 23S rRNA gene that would predict clarithromycin

resistance. Treatment with clarithromycin-based regimens had a lower success rate in patients for whom resistance was predicted (41%) than in patients for whom resistance was not predicted (70%). The study was performed at the Mayo Clinic, and the test is available through their reference laboratory. Another interesting study provided susceptibility data for 345 *H. pylori* isolates from treatment-naïve patients enrolled in a clinical trial evaluating an alternative therapy regimen. The 345 culture-positive isolates were collected from 51 medical centers in 20 states and yielded the following resistance rates: amoxicillin, 6.4%; clarithromycin, 17.4%; metronidazole, 43.6%; rifabutin, 0.0%. In a smaller subset of isolates (71), 2.8% of the isolates were resistant to tetracycline, and 57.8% were resistant to levofloxacin [26].

Neisseria gonorrhoeae is increasingly resistant to recommended antibiotics, and diagnostic testing typically utilizes molecular methods rather than culture. Thus, isolate-specific antimicrobial susceptibility testing is seldom obtained. The Centers for Disease Control and Prevention (CDC) now recommends ceftriaxone with or without azithromycin, abandoning the previously recommended ciprofloxacin due to increased resistance. Klausner et al. developed a molecular assay that can reliably predict ciprofloxacin susceptibility by targeting a single point mutation in *N. gonorrhoeae gyrA* that is responsible for >95% of ciprofloxacin resistance in the organism [27]. Among 211 enrolled subjects for whom remnant specimens were available, 106 subjects had culture-positive infections (some with multiple sites of infection) that tested positive for wild-type *gyrA* serine 91 and were subsequently treated with ciprofloxacin and tested for microbiologic cure 5 to 10 days later. Among the patients who followed the study protocol, 100% demonstrated microbiologic cure. Surely this will find its way to our laboratories sometime in the coming years. What a great clinical study, following up on years of prior work by this group showing that this testing approach was feasible.

Direct from Bottle Blood AST

2021 was a big year for direct disk diffusion (dDD) from positive blood culture to acquire phenotypic antimicrobial susceptibility results faster than by traditional methods. The Clinical and Laboratory Standards Institute (CLSI) added interpretive criteria and methods for *Enterobacterales* dDD to the 31st edition of M100 (expanded to earlier reading times and *Pseudomonas* in the 32nd edition). Savage et al. performed an evaluation of dDD for a large number of Gram-positive and Gram-negative blood culture isolates and also a detailed analysis of the impact of earlier dDD results compared to traditional AST for impact on antibiotic utilization in the form of antibiotic spectrum index (ASI) as a measure of broad- versus narrow-spectrum therapy [28]. They found excellent categorical agreement between dDD and standard AST (VITEK 2), except for clindamycin for *Staphylococcus* spp., and only 31/5,454 (0.6%) VME (19 of which were for clindamycin). They utilized a much broader array of antimicrobials than are included in the M100 method and read results at 18 to 24 h. In response to reporting the Gram stain result, 30% of patients (n = 396) had antibiotics broadened (increased ASI), and 21% had decreased ASI. dDD was reported on average at 24 h after the Gram stain, and in response to dDD, 29% of patients had decreased ASI and 7% had increased ASI. Finally, standard AST was available at 45 h from Gram stain, and at that time, 25% of patients had decreased ASI and only 5% had increased ASI. This is a great study and an innovative graphical approach to visualizing the changes in antimicrobial utilization in response to laboratory results.

Flow cytometry technology has long been an important laboratory diagnostic tool in areas other than microbiology. Silva-Dias et al. described the performance of a flow cytometric approach to rapid susceptibility determination utilizing flow to measure the response of bacterial cells to a 1-h exposure to various antibiotics. Compared to disk diffusion, using CLSI criteria, they reported 96.4% (Gram negative) and 98.6% (Gram positive) categorical agreement, with <1% VME for each [29]. The assay uses a fluorescent probe during antimicrobial exposure to flag bacterial viability and permissivity to fluorescent-molecule uptake to correlate with susceptibility or resistance. It is a very promising approach.

Two studies published in 2021 sought to determine the impact of the Accelerate Pheno system for rapid identification and susceptibility testing on antibiotic utilization and clinical outcomes in patients with Gram-negative bacteremia. Both were large studies (~500 patients in each), one a randomized trial [30] and the other pre-post intervention [31]. Both studies present compelling evidence that this type of assay dramatically improves the time to susceptibility result reporting and leads to earlier changes in antimicrobial therapy, with both narrowing of spectrum and broadening interventions. That's a clear win for antimicrobial stewardship efforts, helping to reduce utilization of broad-spectrum agents and preserve critical antibiotics for cases where they are really needed. Unfortunately, both studies also make it very clear that there is no evidence of benefit to patients whose isolates the test is performed on. There is not even any room to think that larger studies or meta-analysis of many such studies may show such a benefit—there is no trend toward improved mortality or length of stay, etc. A reasonable compromise may be to take the approach described earlier [28] and use direct disk diffusion; not quite as fast but approximately 100 times less expensive.

Susceptibility Testing Updates

Vallabhaneni and colleagues in public health laboratories from New York State and Texas, along with the CDC, evaluated using the AST profile of *Pseudomonas aeruginosa* to predict the presence of carbapenemase-producing isolates [46]. Of 6,192 carbapenem-resistant *P. aeruginosa* (CRPA) isolates from Antimicrobial Resistance network laboratories, only 3% (195) were positive for common carbapenemases (encoded by bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, and bla_{VIM}). In a smaller subset of 965 isolates, only 7 were positive for carbapenemases (carbapenemase-producing CRPA, or CP-CRPA), but the number of isolates needed to test to find one CP-CRPA could be reduced to 64 if only cefepime- or ceftazidime-resistant CRPA isolates were tested and further reduced to 7 if only ceftolozane-tazobactam (C-T)-resistant isolates were tested. This is unlike cefepime/ceftazidime C-T resistance, which was 100% sensitive for CP-CRPA. Previously in our laboratory,

we tested only carbapenem I or R *Enterobacterales* for the presence of bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, and bla_{VIM}. However, this paper was practice changing for us, and our laboratory now also screens C-T-resistant *P. aeruginosa* isolates to find CP-CRPA.

How are you performing AST for *Stenotrophomonas*? This might hurt a little bit. Two studies published in 2021 looked at the performance of commercial automated AST systems [32] or disk diffusion [33] compared to reference broth microdilution for a panel of 109 clinical isolates from bloodstream infections (BSIs). VITEK 2 had <90% categorical agreement (CA) for all drugs tested. In addition, MicroScan and Phoenix performed relatively well for trimethoprim-sulfamethoxazole (TMP-SMX) and minocycline overall (CA for each, >98%); however, each system had 2/9 VME for TMP-SMX. MicroScan and Phoenix also performed poorly for levofloxacin and ceftazidime (CA between 69 and 82%). Based on these data, we should switch to disk diffusion, right? Maybe not. Disk diffusion also performed poorly for ceftazidime, with a 6% VME rate, a 13% ME rate, and only 76% CA. However, disk diffusion did perform relatively well for some key *Stenotrophomonas* drugs, with 95% CA for minocycline and 93% for TMP-SMX, but lower for levofloxacin at 89%, with no VME for these antibiotics. Gradient diffusion performed similarly to disk diffusion [34]. This was also practice changing for our laboratory; we dropped ceftazidime altogether and are considering switching *Stenotrophomonas maltophilia* AST from Phoenix to disk diffusion.

Metallo-β-lactamase (MBL) enzymes are not active against aztreonam, although MBL-harboring organisms often have other β-lactamases that hydrolyze aztreonam. However, these β-lactamases are often inhibited by avibactam. Although aztreonam-avibactam is not yet available clinically (it is in phase III trial) or for laboratory testing materials, several small studies have shown utility of using a combination of aztreonam and ceftazidime-avibactam. So, how do you test if this combination allows activity of the aztreonam? Well, you can arrange to send the isolate to an Antibiotic Resistance network laboratory for broth dilution testing and receive a combination MIC result [35]. Another study evaluated a variety of simple methods that could be employed in most hospital laboratories: disk elution, disk stacking, gradient diffusion strip stacking, and gradient diffusion strip crossing (including different brands of gradient diffusion strips) [36]. The number of isolates tested was low: only 8 with synergy determined by altered broth microdilution fractional inhibitory concentration and 9 additional isolates, a mix of *Enterobacterales* and *P. aeruginosa* with and without MBLs. In this well-defined set of isolates, with three replicates for each counted as unique measures, disk stacking performed poorly, but disk elution was perfect (noting the low *n*) at 100% sensitivity and specificity. Gradient diffusion methods performed relatively well, too, with strip crossing preferable to strip stacking, and MTS (Liofilchem, Italy) strips modestly outperforming E-test strips.

Ceftolozane-tazobactam Resistant *Pseudomonas aeruginosa*

Rubio et al. carried out an interesting in-depth analysis of *P. aeruginosa* isolates from 14 patients that developed resistance to C-T while on therapy, including before and after AST for several β-lactams, as well as sequencing each isolate to find mutations that might have contributed to the developed resistance [37]. Most isolates had mutations in *ampC* or *ampR/ampD*, and this was correlated with decreased activity of ceftazidime-avibactam; however, activity of imipenem-relebactam was maintained, and imipenem activity generally increased. Some isolates had markedly improved activity of piperacillin-tazobactam.

Similarly, Simner et al. studied 16 *P. aeruginosa* isolate pairs from before and after C-T treatment, 10 of which developed resistance to the agent, for activity of cefiderocol, as well as ceftazidime-avibactam and imipenem-relebactam [47]. In only 4 of the 16 isolates did they find increased MICs for cefiderocol (3/4 were still in the susceptible range), and 2/4 had the same E247K mutation in *ampC* (including the non-susceptible isolate). In both isolates with the E247K mutation, C-T and ceftazidime-avibactam MICs were dramatically increased but imipenem-relebactam MICs decreased by three doubling dilutions.

Fournier et al. evaluated 42 unique *P. aeruginosa* isolates with resistance to C-T to determine potential contributing factors in the form of chromosomally encoded or transferable β-lactamases [38]. They were segregated into groups in which carbapenemases were detected, of which IMP, VIM, GES, OXA, and PER-1 enzymes were common, and a second group in which neither carbapenemase nor extended-spectrum beta-lactamase (ESBL) enzymes were detected. The latter group is particularly interesting because it demonstrated massively increased production of *Pseudomonas*-derived cephalosporinase and many isolates with *ampR/ampD* or penicillin-binding protein mutations.

Screening Assays and Antibiotic Utilization

PCR-based methicillin-resistant *Staphylococcus aureus* (MRSA) screening and active antimicrobial stewardship for patients with vancomycin ordered due to suspected pneumonia lead to cost savings outside of the microbiology laboratory and can spare some patient's kidneys from unnecessary vancomycin. Meng et al. performed a pre-post intervention study of total institutional costs associated with nares swab MRSA testing by PCR used to intervene in patients with vancomycin ordered during episodes of suspected pneumonia, including consideration of the costs of labor time for technologists and pharmacists, pharmacy expense for vancomycin, and laboratory reagent costs for both MRSA PCR and vancomycin level testing [39]. They estimated savings of about $40 patient, which is nice enough on its own, but the bigger issue is sparing the patients unnecessary exposure to vancomycin and reduced risk of selective pressure on potential vancomycin-resistant organisms.

Levofloxacin prophylaxis is recommended for neutropenic patients on chemotherapy to prevent Gram-negative bacteremia. Satlin et al. screened 234 such patients for gastrointestinal tract colonization with fluoroquinolone resistant *Enterobacterales* (FQRE) and found 54 patients with FQRE, primarily *Escherichia coli* (91%); of the 54 isolates, 29% also had ESBLs [40]. They screened stool specimens with MacConkey agar supplemented with ciprofloxacin, followed by identification and routine AST. The results were

striking. Of the 54 patients colonized with FQRE on arrival, 17 (31%) developed BSIs with FQRE, and for 15/17, the BSI-causing strain was identical to the colonizing strain, whereas only 2/180 patients without FQRE colonization on admission went on to develop FQRE bacteremia on therapy.

Other Studies

Recently, while evaluating some of our culture protocols and comparing them to procedures from several other academic hospital laboratories, we noted how much variability of practice there is, even for things as common as urine cultures. Prinzi et al. surveyed hospital laboratories that serve pediatric patients for tracheal aspirate (TA) culture practices, including specimen screening by Gram stain; degree of culture workup; which, if any, organisms were ruled out; and when AST was performed [41]. Seventy-three laboratories responded, and the common theme of the survey results was that there was no common theme to the laboratory practices. Forty-four percent of the laboratories had rejection criteria for TA samples based on time from collection and 23% based on Gram stain, and among these, there was not much agreement on rejection criteria (different times or different Gram stain results). How many laboratories always reported *P. aeruginosa* regardless of quantity? Forty-three percent. How many fully identified *S. aureus* if pure or predominant? Fifty-four percent. For most measures evaluated, the percentages of laboratories affirming the practice were >10% and <90%—very little consensus. Obviously, we need more evidence to guide practice in this area.

Why is *Burkholderia pseudomallei* on the select agent list? Gassiep et al. performed a thorough evaluation of 30 technologists exposed to *B. pseudomallei* during routine diagnostic work outside of biosafety cabinets (BSCs) on 1,267 occasions and found that none of them developed infections or became seropositive for antibodies to *B. pseudomallei* [42]. Furthermore, environmental air sampling did not detect aerosolization of *B. pseudomallei* during routine laboratory procedures. We recently had a patient with cultures positive for *B. pseudomallei* [43], and three technologists were exposed outside of a BSC; one of the technologists ended up in the hospital because of it, not for *B. pseudomallei* infection, but rather, an allergic reaction to prophylactic TMP-SMX (none had infections or seroconverted). And then, of course, we followed the select agent rules and did a lot of paperwork. If bad actors really want to acquire *B. pseudomallei*, it would not be that hard; it is readily accessible in nature in certain parts of the world, and it really is not the best organism choice for bioterrorism, either. *Coccidioides* was knocked off the select agent list in 2012. Let's take another one down.

Choi et al. demonstrated that utilization of a multiplex test for meningitis/encephalitis (the Biofire FilmArray ME panel) led to significantly reduced antimicrobial utilization and time to targeted therapy [44]. Additionally, there was a trend (P = 0.03) toward reduced length of stay, although the pre-post intervention study design and lower number of patients (69) in the post-intervention period were probably not powered to evaluate patient outcomes.

Summary

A big thank you is due to the authors of these highlighted studies for completing this important body of work that will enhance our knowledge base, affect our laboratory practices, and ultimately help us better serve patients in 2022 and beyond.

References

[1] Feleke SM, Reichert EN, Mohammed H, Brhane BG, Mekete K, Mamo H, et al. *Plasmodium falciparum* is evolving to escape malaria rapid diagnostic tests in Ethiopia. Nat Microbiol 2021;6:1289-99.

[2] Leeflang MM, Debets-Ossenkopp YJ, Wang J, Visser CE, Scholten RJ, Hooft L, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. Cochrane Database Syst Rev 2015:CD007394.

[3] Theel ES, Doern CD. β-D-Glucan testing is important for diagnosis of invasive fungal infections. J Clin Microbiol 2013;51:3478-83.

[4] White PL, Price JS, Backx M. Evaluation of the performance of the Associates of Cape Cod STAT assay for the diagnosis of invasive fungal disease in critical-care patients with COVID-19. J Clin Microbiol 2021;59:e0086921.

[5] Jani K, McMillen T, Morjaria S, Babady NE. Performance of the sona Aspergillus Galactomannan Lateral Flow Assay in a cancer patient population. J Clin Microbiol 2021;59:e0059821.

[6] Kassis C, Durkin M, Holbrook E, Myers R, Wheat L. Advances in diagnosis of progressive pulmonary and disseminated coccidioidomycosis. Clin Infect Dis 2021;72:968-75.

[7] Grill FJ, Grys TE, Grill MF, Roeder A, Blair JE, Lake DF. Development of a quantitative antigen assay to detect coccidioidal chitinase-1 (CTS1) in human serum. Open Forum Infect Dis 2021;8:ofab344.

[8] Adams E, Quinn M, Tsay S, Poirot E, Chaturvedi S, Southwick K, et al. *Candida auris* in healthcare facilities, New York, USA, 2013-2017. Emerg Infect Dis 2018;24:1816-24.

[9] de Jong AW, Dieleman C, Carbia M, Mohd Tap R, Hagen F. Performance of two novel chromogenic media for the identification of multidrug-resistant *Candida auris* compared with other commercially available formulations. J Clin Microbiol 2021;59.

[10] Das S, Singh S, Tawde Y, Chakrabarti A, Rudramurthy SM, Kaur H, et al. A selective medium for isolation and detection of *Candida auris*, an emerging pathogen. J Clin Microbiol 2021;59.

[11] Lieberman JA, Bryan A, Mays JA, Stephens K, Kurosawa K, Mathias PC, et al. High clinical impact of broad-range fungal PCR in suspected fungal sinusitis. J Clin Microbiol 2021;59:e0095521.

[12] Lamoth F, Akan H, Andes D, Cruciani M, Marchetti O, Ostrosky-Zeichner L, et al. Assessment of the role of 1,3-β-D-glucan testing for the diagnosis of invasive fungal infections in adults. Clin Infect Dis 2021;72:S102-S8.

[13] Badali H, Canete-Gibas C, McCarthy D, Patterson H, Sanders C, David MP, et al. Epidemiology and antifungal susceptibilities of mucoralean fungi in clinical samples from the United States. J Clin Microbiol 2021;59:e0123021.

[14] Wiederhold NP. Antifungal susceptibility testing: a primer for clinicians. Open Forum Infect Dis 2021;8:ofab444.

[15] Desnos-Ollivier M, Lortholary O, Bretagne S, Dromer F. Azole susceptibility profiles of more than 9,000 clinical yeast isolates belonging to 40 common and rare species. Antimicrob Agents Chemother 2021;65.

[16] Wang Z, Zhang L, Zhao M, Wang Y, Bai H, Wang Y, et al. Deep neural networks offer morphologic classification and diagnosis of bacterial vaginosis. J Clin Microbiol 2021;59.

[17] Culbreath K, Piwonka H, Korver J, Noorbakhsh M. Benefits derived from full laboratory automation in microbiology: a tale of four laboratories. J Clin Microbiol 2021;59.

[18] Lo-Ten-Foe JR, de Smet AM, Diederen BM, Kluytmans JA, van Keulen PH. Comparative evaluation of the VITEK 2, disk diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* strains. Antimicrob Agents Chemother 2007;51:3726-30.

[19] Kabir S, Rahman SMM, Ahmed S, Islam MS, Banu RS, Shewade HD, et al. Xpert Ultra assay on stool to diagnose pulmonary tuberculosis in children. Clin Infect Dis 2021;73:226-34.

[20] Brown-Elliott BA, Woods GL. Antimycobacterial susceptibility testing of nontuberculous mycobacteria. J Clin Microbiol 2019;57.

[21] Marras SAE, Chen L, Shashkina E, Davidson RM, Strong M, Daley CL, et al. A molecular-beacon-based multiplex real-time PCR assay to distinguish *Mycobacterium abscessus* subspecies and determine macrolide susceptibility. J Clin Microbiol 2021;59:e0045521.

[22] Ward JD, Cornaby C, Schmitz JL. Indeterminate QuantiFERON Gold Plus results reveal deficient interferon gamma responses in severely ill COVID-19 patients. J Clin Microbiol 2021;59:e0081121.

[23] Coppock D, Zurlo CE, Meloni JM, Goss SL, Zurlo JJ, Pettengill MA. Interferon gamma release assay mitogen responses in COVID-19. Infect Dis Clin Pract 2022;30:e1085.

[24] Wikell A, Jonsson J, Dyrdak R, Henningsson AJ, Eringfalt A, Kjerstadius T, et al. The impact of borderline Quantiferon-TB Gold Plus results for latent tuberculosis screening under routine conditions in a low-endemicity setting. J Clin Microbiol 2021;59:e0137021.

[25] Marrero Rolon R, Cunningham SA, Mandrekar JN, Polo ET, Patel R. Clinical evaluation of a real-time PCR assay for simultaneous detection of *Helicobacter pylori* and genotypic markers of clarithromycin resistance directly from stool. J Clin Microbiol 2021;59.

[26] Hulten KG, Lamberth LB, Kalfus IN, Graham DY. National and regional US antibiotic resistance to *Helicobacter pylori*: lessons from a clinical trial. Gastroenterology 2021;161:342-4 e1.

[27] Klausner JD, Bristow CC, Soge OO, Shahkolahi A, Waymer T, Bolan RK, et al. Resistance-guided treatment of gonorrhea: a prospective clinical study. Clin Infect Dis 2021;73:298-303.

[28] Savage TJ, Rao S, Joerger J, Ozonoff A, McAdam AJ, Sandora TJ. Predictive value of direct disk diffusion testing from positive blood cultures in a children's hospital and its utility in antimicrobial stewardship. J Clin Microbiol 2021;59.

[29] Silva-Dias A, Perez-Viso B, Martins-Oliveira I, Gomes R, Rodrigues AG, Canton R, et al. Evaluation of FASTinov ultrarapid flow cytometry antimicrobial susceptibility testing directly from positive blood cultures. J Clin Microbiol 2021;59:e0054421.

[30] Banerjee R, Komarow L, Virk A, Rajapakse N, Schuetz AN, Dylla B, et al. Randomized trial evaluating clinical impact of RAPid IDentification and susceptibility testing for Gram-negative bacteremia: RAPIDS-GN. Clin Infect Dis 2021;73:e39-e46.

[31] Robinson ED, Stilwell AM, Attai AE, Donohue LE, Shah MD, Hill BK, et al. Implementation of a rapid phenotypic susceptibility platform for Gram-negative bloodstream infections with paired antimicrobial stewardship intervention: is the juice worth the squeeze? Clin Infect Dis 2021;73:783-92.

[32] Khan A, Arias CA, Abbott A, Dien Bard J, Bhatti MM, Humphries RM. Evaluation of the Vitek 2, Phoenix, and MicroScan for antimicrobial susceptibility testing of *Stenotrophomonas maltophilia*. J Clin Microbiol 2021;59:e0065421.

[33] Khan A, Pettaway C, Dien Bard J, Arias CA, Bhatti MM, Humphries RM. Evaluation of the performance of manual antimicrobial susceptibility testing methods and disk breakpoints for *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2021.

[34] Khan A, Pettaway CH, Dien Bard J, Arias CA, Bhatti MM, Humphries RM. Erratum for Khan et al. "Evaluation of the Performance of Manual Antimicrobial Susceptibility Testing Methods and Disk Breakpoints for *Stenotrophomonas maltophilia*". Antimicrob Agents Chemother 2021;65.

[35] Bhatnagar A, Boyd S, Sabour S, Bodnar J, Nazarian E, Peinovich N, et al. Aztreonam-avibactam susceptibility testing program for metallo-beta-lactamase-producing *Enterobacterales* in the Antibiotic Resistance Laboratory network, March 2019 to December 2020. Antimicrob Agents Chemother 2021;65:e0048621.

[36] Khan A, Erickson SG, Pettaway C, Arias CA, Miller WR, Bhatti MM. Evaluation of susceptibility testing methods for aztreonam and ceftazidime-avibactam combination therapy on extensively drug-resistant Gram-negative organisms. Antimicrob Agents Chemother 2021;65:e0084621.

[37] Rubio AM, Kline EG, Jones CE, Chen L, Kreiswirth BN, Nguyen MH, et al. *In vitro* susceptibility of multidrug-resistant *Pseudomonas aeruginosa* following treatment-emergent resistance to ceftolozane-tazobactam. Antimicrob Agents Chemother 2021;65.

[38] Fournier D, Carriere R, Bour M, Grisot E, Triponney P, Muller C, et al. Mechanisms of resistance to ceftolozane/tazobactam in *Pseudomonas aeruginosa*: results of the GERPA multicenter study. Antimicrob Agents Chemother 2021;65.

[39] Meng L, Pourali S, Hitchcock MM, Ha DR, Mui E, Alegria W, et al. Discontinuation patterns and cost avoidance of a pharmacist-driven methicillin-resistant *Staphylococcus aureus* nasal polymerase chain reaction testing protocol for de-escalation of empiric vancomycin for suspected pneumonia. Open Forum Infect Dis 2021;8:ofab099.

[40] Satlin MJ, Chen L, Douglass C, Hovan M, Davidson E, Soave R, et al. Colonization with fluoroquinolone-resistant *Enterobacterales* decreases the effectiveness of fluoroquinolone prophylaxis in hematopoietic cell transplant recipients. Clin Infect Dis 2021;73:1257-65.

[41] Prinzi AM, Parker SK, Curtis DJ, Ziniel SI. The Pediatric Endotracheal Aspirate Culture Survey (PETACS): examining practice variation across pediatric microbiology laboratories in the United States. J Clin Microbiol 2021;59.

[42] Gassiep I, Bauer MJ, Harris PNA, Chatfield MD, Norton R. Laboratory safety: handling *Burkholderia pseudomallei* isolates without a biosafety cabinet. J Clin Microbiol 2021;59:e0042421.

[43] Arya A, Shaikh H, Weber D, Pettengill M, Moss S. Fever in a returning traveler: a case and literature review of melioidosis. IDCases 2021;26:e01340.

[44] Choi JJ, Westblade LF, Gottesdiener LS, Liang K, Li HA, Wehmeyer GT, et al. Impact of a multiplex polymerase chain reaction panel on duration of empiric antibiotic therapy in suspected bacterial meningitis. Open Forum Infect Dis 2021;8:ofab467.

[45] Cherkaoui A, Renzi G, Vuillenumier N, Schrenzel J. Performance of fully automated antimicrobial disk diffusion susceptbiility testing using Copan WASP Colibri coupled to the Radian In-Line Carousel and Expert Sysetem. J Clin Microbiol. 2021;59(9):e0077721.

[46] Vallabhaneni S, Huang JY, Grass JE, Bhatnagar A, Sabour S, Lutgring JD, et al. Antimicrobial susceptibility profiles to predict the presence of carbapenemase genes among carbapenem-resistant *Pseudomonas aeruginosa* isolates. J Clin Microbiol. 2021;59(6): e02874-20.

[47] Simner PJ, Beisken S, Bergman Y, Posch AE, Cosgrove SE, Tamma PD. Cefiderocol activity against clinical *Pseudomonas aeruginosa* isolates exhibiting ceftolozane-tazobactam resistance. Open Forum Infect Dis. 2021;8(7):ofab311.