PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
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AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

Happy New Year! Looking back on 2016, our division had another productive year supporting multiple AACC strategic initiatives. I was pleased that we received a robust response to a call for nominations to serve on the PMF board. Though there were limited spots up for election, we look forward to engaging more of our members in the division's activities beginning in 2017. These include:

- Developing a pediatric-focused education curriculum and associated materials.
- Collaborating with the History division to document the history of the PMF division.
- Working with AACC staff to refine the pediatric reference intervals initiative.

Please consider volunteering your time and expertise to assist us in one (or more) of these areas. Feel free to contact me with your interest or any questions and other ideas.

In this issue, we continue our ABC's of Laboratory Medicine with letter 'Y', focused on 'Yersinia.' The 'Interview with a Colleague' segment features incoming President, Dr. Michael J. Bennett, who is a long-time PMF member and recognized expert in pediatric laboratory medicine. Dr. Kelly Doyle summarizes newly published research results that may help identify diagnostic approaches as well as therapeutic treatments to delay membrane rupture and pre-term birth. Finally, also in this edition: the election results are in! Find out who will serve as our newest division officers.

I hope that you find this edition of the newsletter informative and that you consider contributing to our efforts to advance the practice and science of pediatric and maternal fetal laboratory medicine.

Shannon Haymond, PhD
Chair, AACC PMF Division

TABLE OF CONTENTS

THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

Y IS FOR "YERSINIA"

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The genus *Yersinia* contains 19 species (1), of which three are pathogenic in humans: *Yersinia enterocolitica*, *Yersinia pseudotuberculosis* and *Yersinia pestis.* *Yersinia* species are members of *Enterobacteriaceae* but are slower growing than other *Enterobacteriaceae* when grown under standard incubation conditions, and therefore will be overgrown or outcompeted in non-sterile specimens, especially stool cultures.

Y. enterocolitica causes gastroenteritis associated with contaminated food or water and is a reportable illness. The Centers for Disease Control (CDC) estimates that *Y. enterocolitica* causes 117,000 infections, 640 hospitalizations and 35 deaths annually in the US (2). Infection with *Y. enterocolitica* occurs most often in young children and is most common during winter months (3). *Y. enterocolitica* is associated with swine carriage; ingestion of undercooked pork, especially chitterlings (chitlins), is the primary source of illness. Disease most commonly occurs during the winter months due to the association with chitlins, which are traditionally prepared during the holiday season. Symptoms of gastroenteritis caused by *Y. enterocolitica* include fever, abdominal pain and diarrhea, which is often bloody. Most cases of gastroenteritis are self-limiting and do not require antimicrobial therapy. However, immunocompromised patients are at risk of developing subsequent systemic illness and may require treatment. Antimicrobial options include fluoroquinolones, trimethoprim-sulfamethoxazole and expanded-spectrum cephalosporins. Routine stool cultures typically do not detect *Y. enterocolitica* due to its slower growth rate compared to enteric flora, and most hospitals offer a separate *Yersinia* stool culture order. If *Y. enterocolitica* is suspected, the microbiology laboratory should be alerted or a *Yersinia* culture ordered. When *Y. enterocolitica* is suspected, stool specimens are inoculated to a *Yersinia* selective agar, typically cefsulodin-irgasan-novobiocin (CIN) agar, which is incubated at room temperature. *Yersinia* species appear as bullseye colonies on CIN, which have a red center and clear border (Fig 1).

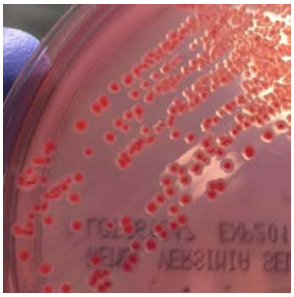

Fig 1. Characteristic *Yersinia* bullseye colonies on CIN agar.

Because *Y. enterocolitica* is not detected on routine stool cultures, its incidence is likely underestimated. Two commercial molecular gastrointestinal panels, the Verigene Enteric Pathogens Test (Luminex Corporation, Austin, TX) and the BioFire Gastrointestinal Pathogens Panel (BioFire Diagnostics, Salt Lake City, UT), include *Y. enterocolitica* as a target. As molecular gastrointestinal pathogen panels are more widely adopted, they may provide a better estimate of the incidence of *Y. enterocolitica* gastroenteritis in the US. At Cook Children's Medical Center, approximately 3,000 routine stool cultures are performed annually whereas only approximately 30 *Yersinia* stool cultures are performed each year. All of the *Yersinia* stool cultures performed in the last ten years were negative. However, Cook Children's implemented the Verigene Enteric Panel in December 2016, replacing traditional stool culture. Since implementation, *Y. enterocolitica* has been detected in two patients, both of which were confirmed when the specimens were cultured to Yersinia Selective Agar (CIN; Remel, Lenexa, KS). Per the physicians, neither patient would have had a *Yersinia* stool culture ordered, and the diagnosis would have been missed. One patient was seen in the ED

and discharged without questioning about potential food exposures. The other patient was admitted and was treated due to his age and immune status. Only after the test was reported as being positive was the family questioned about exposure to chitlins, and it was discovered that chitlins had recently been prepared on the same kitchen countertop where the child's formula was prepared.

Y. pseudotuberculosis is the least common of the three *Yersinia* species that cause infections in humans. It causes gastroenteritis, accompanied by fever and abdominal pain, but it is not usually associated with diarrhea. *Y. pseudotuberculosis* also causes mesenteric lymphadenitis. During a 12 year period (1996-2007), the CDC reported only 18 cases of *Y. pseudotuberculosis* nationwide (4). In contrast to *Y. enterocolitica*, the majority of *Y. pseudotuberculosis* infections were found to occur in adults and most commonly in white males (4). *Y. pseudotuberculosis* was also more commonly isolated from invasive sites, with 12 of 18 isolates being recovered from blood, while only a single isolate was recovered from stool (4).

Y. pestis is the most well-known *Yersinia* species due to its role as the causative agent of plague, made infamous by the Black Death of the 14th century. Presently, the majority of worldwide cases of plague occur in Africa, while approximately 1% of cases occur in the US (3). Most cases of *Y. pestis* in the US occur in the four corners region (Colorado, New Mexico, Arizona and Utah), with more than 80% of cases occurring in Colorado, New Mexico and Arizona (5). Other cases are scattered throughout California and Oregon. In contrast to historical urban epidemics, cases are now primarily scattered throughout rural areas.

Three forms of plague exist: bubonic, septicemic and pneumonic and all have high mortality rates if left untreated (3). Bubonic plague is characterized by buboes, or swollen lymph nodes, and is vectored by rat fleas, which may be brought into the home by pet dogs or cats. Septicemic plague develops after the bite of any infected flea or after handling infected animals. Pneumonic plague is contracted through inhalation of infectious respiratory droplets from infected humans or cats, which are particularly susceptible to plague. Pneumonic plague may also develop subsequent to untreated bubonic or septicemic plague.

Y. pestis is classified as a category A biothreat agent due to the properties that make it a good bioweapon, which include wide availability, potential for mass production and aerosolization, contagious nature and potential to cause high fatality rates. The first reported use of *Y. pestis* as a bioweapon was in the 14th century, when the Tartars, invading the city of Kaffa, catapulted bodies of plague victims over the city walls, resulting in an epidemic (6). *Y. pestis* appears as nondescript colonies on blood agar, although colonies may have a "fried egg" appearance, and is a non-fermenter on MacConkey agar. It is the only *Yersinia* species that is non-motile at room temperature. Because of its status as a biothreat agent, suspected cases must be reported to the local public health entity and the bacterial isolate sent for confirmatory testing.

Although *Yersinia* species other than *Y. enterocolitica, Y. pseudotuberculosis* and *Y. pestis* are considered to be non-pathogenic in humans, they have been isolated in stool cultures from patients with gastrointestinal symptoms with no alternative diagnosis (7). It is possible that these species are pathogens but are underrecognized due to the limitations of current culture methods.

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Interview with a Distinguished Colleague

Michael J. Bennett, PhD, FRCPath, DABCC, FACB. 2017 AACC President.
Professor of Pathology and Laboratory Medicine, Director of the Metabolic Disease Laboratory. Children's Hospital of Philadelphia. University of Pennsylvania, Philadelphia, Pennsylvania, USA

What are you most looking forward to in your year as president of AACC?

Being elected president was the culmination of my career to date as a clinical chemist. Earlier this year I was asked to be on a taskforce to develop strategic initiatives for the association for the next three years. I'm very much looking forward to working with the board of directors and AACC staff in completing those initiatives. As a whole the initiatives will provide even better educational programs, increase the visibility of our profession and provide advocacy for many of our projects.

I'm also looking forward to interacting with as many members of the association as possible in 2017. The AACC President typically is invited to many of the local sections to both give a talk and also to meet members within their own local sections to hear what works well and where improvements might be made. My year in 2017 is already filling up with invitations.

There are also many opportunities for the president to meet leaders of other similar organizations to discuss areas in which we might collaborate. Many of these meetings are set up at the annual meeting where the president hosts visitors representing those organizations. You would be surprised to know how few CE credits the president actually receives at the annual meeting due to these other commitments. However, I am looking forward to these interactions.

What development would you like to see occur in pediatric laboratory medicine over the next 3 years?

One of the major areas of advocacy on the association's agenda is that of pediatric reference intervals. I was involved with the development of the policy statement recently put out by AACC on reference intervals. I would like to see this initiative go one step further to a practical level where a repository is identified to hold and store samples from normal pediatric patients for reference interval determinations. We are in touch with the CDC and NIH in this matter and I would like to see a practical solution to the problem.

At a global level, one of my personal projects as president is to help develop newborn screening programs in developing counties. With the help of the Latin American Workgroup of AACC, we are planning a workshop in Colombia in 2017 to investigate and promote building a universal program in that country. In three years, I would like to see this program come to fruition.

Analytically, but less optimistically, I would like to see a major one or more of our vendor partners seriously develop automated analyzers that can readily handle pediatric samples. This has been an unresolved issue for decades but maybe as president of AACC and a pediatric background, the vendors will listen.

What do you see as the biggest challenges facing laboratorians in the next several years?

Keeping up with the pace of technological developments (mass spectrometry, molecular diagnostics, point of care). Embracing these changes, but also recognizing that they often take place so quickly that keeping up is

problematic. Next gen is typically already last gen.

There is a tremendous growth in our knowledge base and keeping up is problematic. In order to retain our visibility as a profession we need to be able to be incredibly well-educated and current.

Visibility has always been an issue as we generally practice our skills away from direct patient care. We have always, and still do need to be seen as the experts that we are. In pediatric laboratory medicine, we have many opportunities to be seen as the experts as very few of our clinical colleagues could interpret, for example, an organic acid chromatogram without our input. We need to participate in clinical rounds and conferences to promote our visibility.

Identifying opportunities to specialize in laboratory medicine. We need to find the time to develop expert skills in a particular area of laboratory medicine. All too often, this time is not available due to clinical load. Somehow though, we really do need opportunity to grow and this can pose a challenge.

Excerpt from the Literature

Kelly Doyle, PhD, DABCC, Clinical Chemist, Intermountain Healthcare, Salt Lake City, UT, USA

Calciprotein particles as potential etiologic agents of idiopathic preterm birth

Lydia L. Shook, Catalin S. Buhimschi, Antonette T. Dulay, Megan E. McCarthy, John T. Hardy, Christina M. Duzyj Buniak, Guomao Zhao, Irina A. Buhimschi. Sci Transl Med, 2016. **8**(364): p. 364ra154.

According to the National Institute of Child Health and Human Development, “preterm birth (PTB) is the most common cause of infant death and is the leading cause of long-term disability related to the nervous system in children.” However, mothers at risk of idiopathic preterm labor are difficult to identify due to a paucity of knowledge around underlying pathophysiology of PTB.

Recently, a team of researchers at Nationwide Children’s Hospital in Columbus, OH describe in their basic research article how they may have discovered an underlying etiology of preterm premature rupture of membrane (PPROM)-mediated PTB based on ectopic calcification. Specifically, they observed an elevated frequency of calcium-protein conglomerates, called calciprotein particles (CPPs) in amniochorion tissues and correlated decrease of protein fetuin-A in amniotic fluid of women experiencing idiopathic PPROM. Fetuin-A acts as a mineralization inhibitor but was found to be the major protein constituent in the CPPs. Interestingly, the sequestration of fetuin-A in CPPs, may create an environment within the amniotic fluid that is depleted of essential proteins and minerals as well as increase the activity of prostaglandin-E2 which is a well-documented mediator of labor induction and membrane rupture.

From a diagnostics perspective, calciprotein or fetuin-A concentrations may be potential markers used to identify women at risk of idiopathic PTB, but clinical utility is complicated by the fact that there is not a direct correlation to concentrations of fetuin-A in maternal serum or fetal cord-blood as demonstrated in the article.

Yet unknown are the triggers of CPP formation and decrease protein fetuin-A concentrations but these discoveries may help identify diagnostic approaches as well as therapeutic treatments to delay membrane rupture and PTB.

Welcome to our new PMF Division Board Members

The foundation of any vibrant organization is built on the generosity of those who volunteer their time and skills to serve their community. Please help us in welcoming our new PMF Board Members for 2017:

Treasurer:

- **Angela Ferguson, PhD**. Children's Mercy, University of Missouri-Kansas City School of Medicine, Kansas City, MO

Members at Large:

- **Mark Kellogg, PhD**. Children's Hospital Boston, Harvard Medical School, Boston, MA
- **Joesph Wiencek, PhD.** Vanderbilt University, Nashville, TN

Newsletter Editorial Board:

- **Kelly Doyle, PhD.** Intermountain Healthcare, Salt Lake City, UT

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