# Ways to move quickly on bloodstream infection

## William Check, PhD

October 2013—Since 1942, when penicillin was first used to treat infections caused by gram-positive bacteria, many improved and potent beta-lactam antimicrobials have been developed. Yet today, if a patient in an intensive care unit develops a bloodstream infection with *Staphylococcus aureus*, that person has a one in three chance of dying. High mortality rates apply to many other pathogens that cause bloodstream infections in ICU patients—from one in five for coagulase-negative staphylococci and *Escherichia coli* to almost 40 percent for *Pseudomonas aeruginosa* and *Candida* spp. *Enterobacter* spp and *Enterococcus* spp have intermediate mortality rates: one in four and one in three, respectively. Even among patients on a non-ICU ward, bloodstream infections are associated with mortality rates between 20 percent and 30 percent.

At this year's annual meeting of the American Society for Microbiology, Christine C. Ginocchio, PhD, MT(ASCP), senior medical director and chief of the Division of Infectious Disease Diagnostics at North Shore-LIJ Laboratories, gave the Division C Lecture on the clinical and infection control implications of rapid detection and identification of bloodstream pathogens. Recently introduced molecular technologies can significantly shorten time to identification. Technologies in development now may shorten that time even more.

**Dr. Ginocchio**

In her ASM lecture, which was the BD Award for Research in Clinical Microbiology presentation, Dr. Ginocchio showed that, at an incidence of 377 per 100,000 population, sepsis is more common than stroke (223), cancer (332), or heart disease (208) (Hall MJ, et al. NCHS data brief, No. 62. Hyattsville, Md. National Center for Health Statistics, 2011).

Moreover, the NCHS document says the rate of sepsis is increasing. Septicemia or sepsis as a first-listed diagnosis more than doubled between 2000 and 2010, from 11.6 per 10,000 population to 26.5, says Dr. Ginocchio, who is professor in the Departments of Pathology and Laboratory Medicine and of Molecular Medicine in the Feinstein Institute for Medical Research, Hofstra North Shore-LIJ School of Medicine, New York. Hospitalizations did not increase during that period.

Among patients hospitalized for sepsis, length of stay rises from 4.8 to 8.4 days. Particularly affected are those under age 65, whose LOS rises from 4.3 to 9.1 days when sepsis is present, according to the NCHS.

In addition to the toll it takes on patients, sepsis is costly. "Patients can get sepsis as a result of nosocomial infection, which is no longer reimbursable," Dr. Ginocchio points out.

Time to organism identification and antimicrobial susceptibility testing is critical to improving survival, as demonstrated by data showing a strong inverse relationship between time to effective antibiotic therapy and survival. In one patient analysis, by four hours half of patients had received effective antibiotic therapy. Yet even with this early treatment only half survived (Kumar A, et al. *Crit Care Med*. 2006;34: 1589–1596).

Dr. Ginocchio discussed currently available rapid molecular methods for directly detecting organisms from positive blood cultures.

One, AdvanDx PNA FISH, is a technology in which fluorescence-tagged peptide nucleic acid (PNA) probes detect 16S rRNA directly from positive blood cultures. “One of the problems with the old version of these assays was that it took many steps and many hours,” Dr. Ginocchio said in an interview. QuickFISH is the streamlined version; it has a turnaround time of 20 minutes once the blood culture turns positive, she says. Four kits are available: *S. aureus* versus coagulase-negative staphylococci; *E. faecalis* versus non-*faecalis* enterococci (E. *faecium*); *E. coli* versus *K. pneumoniae* versus *P. aeruginosa*; and *C. albicans* versus *C. parapsilosis* versus *C. glabrata*. Speciation of *Candida* helps decide whether to treat with fluconazole.

Dr. Ginocchio showed several published studies demonstrating shorter time to identification with PNA FISH. One retrospective analysis found a potential savings of 60 to 80 hours relative to standard culture methods for identifying a range of organisms that cause bloodstream infection (Harris DM, Hata DJ. *Ann Clin Microbiol Antimicrob*. 2013;12:2). Accuracy was 99 percent.

A second study, using PNA FISH to distinguish *S. aureus* from coagulase-negative staphylococci in blood cultures, demonstrated reduced length of stay (from six to four days) with PNA FISH (Forrest GN, et al. *J Antimicrob Chemother*. 2006;58:154–158). No significant effect on vancomycin usage was found.

In a third bloodstream infection study, comparing PNA FISH to the *C. albicans* screen test for differentiating *C. albicans* from non-albicans *Candida* species, potential cost savings were identified (Alexander BD, et al. *Diagn Microbiol Infect Dis*. 2006;54:277–282). Savings were realized through a decrease in antifungal drug costs, particularly caspofungin, the authors wrote. They noted that their interpretation assumed “physician notification of yeast identity concurrent with blood culture positivity.”

Turning to amplification methods, Dr. Ginocchio listed four: BD-GeneOhm’s StaphSR, Cepheid’s Xpert MRSA/SA (methicillin-resistant and -sensitive *S. aureus*), Nanosphere’s Verigene system for gram-negative and -positive organisms, and BioFire’s assay for bloodstream infection. All work on samples from a positive blood culture bottle. “There are no FDA-cleared tests that can be done directly from blood,” she says.

BD-GeneOhm’s StaphSR yields results within two hours. Results are reported as negative or positive for *S. aureus* or MRSA or both.

Cepheid’s GeneXpert MRSA/SA was approved in August. In one study, outcomes during the four-month period after the assay was adopted were compared with the four months before its adoption. Results of the assay were communicated to an infectious disease pharmacist for possible regimen change. Investigators concluded that it “allows rapid differentiation of *S. aureus* bacteremia, enabling timely, effective therapy and is associated with decreased length of stay and health care costs” (Bauer KA, et al. *Clin Infect Dis*. 2010;51:1074–1080). Its impact was more pronounced on MSSA isolates, increasing the number of switches to non-vancomycin therapy and shortening the time to switching.

BioFire’s amplification assay for bloodstream infection was also recently approved, in this case for a range of gram-positive and -negative organisms and *Candida* species.

Nanosphere’s Verigene system performs multiplex analysis using one cartridge per specimen. It can also detect three genetic resistance determinants. Results from each test are available in about 1.5 hours. The Verigene BC-GP is FDA cleared for detection of 12 gram-positive bloodstream pathogens. In a report that appeared online in July, Dr. Ginocchio and other investigators conducted a five-center study to evaluate the diagnostic accuracy of the Verigene BC-GP directly from positive blood culture broths containing gram-positive bacteria (Buchan BW, et al. *PLoS Med*. 2013; Jul;10: e1001478).

They found:

- Sensitivity was 94.8 percent to 100 percent, except for *E. faecium,* 92.6 percent.

- Specificity was 98.9 percent to 100 percent.
- Sensitivity for *mecA* and *vanA* genes was 98.6 percent and 100 percent, respectively.
- Identification was achieved an average of 42 to 47 hours sooner using the BC-GP test compared with routine culture methods; for the *Strep viridans* group, it was 70 hours sooner.
- Retrospective analysis of 107 separate blood cultures demonstrated that identification of methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus* spp was completed an average of 41.8 to 42.4 hours earlier compared with routine culture methods.

One drawback: About 7.5 percent of single-organism cultures contained gram-positive bacteria not present on the BC-GP test panel.

A version of the Verigene BC-GP for gram-negative bloodstream infection is in development.

Dr. Ginocchio was part of a group that evaluated a detection method based on RNA-dependent nucleic acid sequence-based amplification and molecular beacon (NASBA-MB) that uses pan-gram-negative, pan-gram-positive, pan-fungal, pan-*Candida*, and pan-*Aspergillus* probes and primers (Zhao Y, et al. *J Clin Microbiol*. 2009;47:2067–2078). In a clinical study of 570 blood culture samples, the candidate assay produced "excellent" results, Dr. Ginocchio says. However, on further evaluation, it did not have adequate sensitivity to detect pathogens directly from patient samples. "We could get a faster result if we sampled blood culture bottles every few hours," Dr. Ginocchio says, "but this is not practical."

Radically different from molecular methods is Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry. In MALDI-TOF MS, microbes are identified by matching the protein profiles of sample organisms generated via the procedure to profiles contained in a proprietary database. Like molecular methods, MALDI-TOF MS is used on positive blood culture bottles. However, since it does not require an amplification step, it is even more rapid, returning results for each sample in minutes.

Dr. Ginocchio characterizes MALDI-TOF MS as fast, accurate, comprehensive, and inexpensive. "It is no more expensive than the instruments we use for traditional identification," she says. Vitek, Phoenix, and MicroScan automated identification instruments cost about $150,000; a mass spectrometer can be purchased for under $200,000. More significant, reagents for conventional machines cost about $5 to $8 and for molecular assays about $75 to $125 per test, while per-test cost for a mass spectrometer is less than $1.

Other advantages of MALDI-TOF MS are that it is not necessary to predetermine whether an organism is gram-positive or gram-negative and it has the potential for future susceptibility testing.

MALDI-TOF MS can have some difficulty differentiating very similar organisms such as *Shigella* spp from *E. coli*. Some systems have difficulty with differentiating *Streptococcus pneumoniae* and *S. mitis/oralis*. However, "The Vitek MS can very accurately differentiate these organisms," Dr. Ginocchio says.

Several studies evaluating the use of MALDI-TOF MS for the identification of bacteria and yeast from positive blood culture bottles have been published. Using one of the two major instruments, the Bruker BioTyper, a Dutch group analyzed 101 positive BacT/Alert bottles. Correct identification at the species level was obtained in 78 percent of specimens—64 percent of gram-positive cocci and 96 percent of gram-negative rods (Loonen AJ, et al. *Eur J Clin Microbiol Infect Dis*. 2012;31:1575–1583). In a similar study using the BioMérieux Vitek MS system, a group at Emory University was able to obtain a correct identification to species level in 73 percent of 259 bottles (Fothergill A, et al. *J Clin Microbiol*. 2013;51: 805–809).

Another Dutch group showed that incorporating MALDI-TOF MS into the identification system increased appropriate antibiotic prescribing. Using an alternate-month protocol, they found that, in the months when MALDI-TOF MS was part of the system, time to species identification was reduced by almost 30 hours. In addition, there was an 11.3 percent increase in the proportion of patients receiving appropriate antibiotic treatment 24 hours after blood culture positivity (64 percent in the control period versus 75.3 percent in the intervention period) (Vlek AL, et al. *PLoS One*. 2012;7:e32589).

A Swiss group found that MALDI-TOF MS had a positive impact on more cases than did Gram stain. Using impact on empirical antibiotic choice as the primary outcome, they found that, among 202 episodes of gram-negative bacteremia, Gram stain reporting had an impact in 20.8 percent of cases, while MALDI-TOF MS identification led to a modification of empirical therapy in 35.1 percent. The most frequently observed impact was an early appropriate broadening of the antibiotic spectrum, the investigators reported (Clerc O, et al. *Clin Infect Dis*. 2013; 56:1101–1107).

A five-center group evaluated the accuracy of Vitek MS for bacteria and yeast identification from colonies, using 16S ribosomal RNA gene sequencing and biochemical testing as the comparators.

Results for species-level identification were as follows:

- Gram-positive cocci: 92.8 percent (Rychert J, et al. *J Clin Microbiol*. 2013;51:2225–2231).
- Yeast: 96.1 percent (Westblade LF, et al. *J Clin Microbiol*. 2013;51: 2267–2272).
- *Enterobacteriaceae*: 83.8 percent (Richter SS, et al. *Eur J Clin Microbiol Infect Dis*. July 2, 2013, Epub ahead of print).
- non-*Enterobacteriaceae* gram-negative bacilli: 77.8 percent (Manji R, et al. *Eur J Clin Microbiol Infect Dis*. Sept. 10, 2013, Epub ahead of print).

Based in large part on these data, in August the FDA gave the Vitek MS 510(k) de novo clearance, the first such platform approved for clinical use in the United States.

Dr. Ginocchio was part of the five-center group that conducted the clinical trials for the Vitek MS. Her laboratory was validating it for clinical use and monitoring its performance while the application was under consideration at the FDA. “It performed quite excellently. You do not have to do extraction or up-front preparation prior to applying the organisms to the target slide. For bacteria, matrix is then added to the dried spot; for yeast, a drop of formic acid is added to the target slide prior to the addition of matrix,” she says.

With the FDA now having cleared the instrument, her laboratory will soon use it clinically. “We plan to switch to Vitek MS as the primary method for microbial identification.” Not all organisms will be identified, and supplemental tests may be required, she says. “However, for the 90-plus percent of the most frequently isolated aerobic and anaerobic bacteria and yeast, MALDI-TOF MS should provide a rapid and accurate identification.” Culture that would routinely take 48 to 72 hours can now be reported in 24 to 36 hours when combined with a rapid automated susceptibility test, she says.

Identification alone can provide initial critical information for guiding antimicrobial therapy for such pathogens as *Pseudomonas, Acinetobacter, Stenotrophomonas*, and fluconazole-resistant yeast, including *Candida krusei*.

“The direct identification of pathogens from positive blood cultures will allow a rapid assessment of the potential efficacy of empiric therapy and guide change in a time frame that should have a significant impact on patient morbidity and mortality,” Dr. Ginocchio says.

One issue she identified is the need to validate MALDI-TOF MS on positive blood cultures as an off-label use, since it is FDA cleared for identification of bacteria or yeast colonies from culture plates. “We have just completed our in-house validation on 1,000 positive blood cultures,” she says.

MALDI-TOF MS will miss mixed infections or not identify the predominant organism, she says.

Specific Technologies’ colorimetric sensor array technology was the last method for use on positive blood cultures that Dr. Ginocchio described. By sampling volatile organic compounds in the headspace of the culture, this technique produces an organism-specific difference map that can serve as a profile for identification. In one experiment Dr. Ginocchio illustrated, the Specific Technologies sensor identified organisms to the species level greater than 20 percent faster than the BioMérieux BacT/Alert 3D system gave a CO2 positive growth signal only.

Identifying pathogens directly from patient blood samples without having to culture would be the fastest way to diagnose bloodstream infection. While no method for this use is approved in the United States, Dr. Ginocchio spoke about the possibilities.

Although the Roche LightCycler SeptiFast Test is available for clinical use in Europe, Dr. Ginocchio told CAP TODAY that it was not going to be brought to the FDA because its sensitivity is not sufficiently high to meet FDA specifications. “It works well in children and newborns for sepsis because they have a high bacterial burden,” she says.

A product with promising sensitivity is T2 Biosystems’ T2MR assay. In this assay, microbial cells are lysed, followed by PCR and hybridization of the amplified pathogen DNA to capture probe-decorated nanoparticles. Hybridization with the superparamagnetic nanoparticles yields nanoparticle microclusters that cause large changes in the sample’s T2MR signal. Spiked human blood samples were used to show that the method detects five *Candida* species with a limit of detection as low as 1 CFU/mL—though for four of the species sensitivity was acceptable only at 2 to 3 CFU/mL—and a time to result of less than three hours (Neely LA, et al. *Sci Transl Med*. 2013; Apr 24;5[182] 182ra54). Agreement with blood culture was 97.8 percent for positive samples and 100 percent for negative samples.

“This method has the sensitivity you would need to work directly from whole blood patient samples. You can do it with any organism; *Candida* is just the first one they tried,” she says, adding that the company plans to develop versions for other organisms.

In September, T2 Biosystems announced the initiation of the direcT2 clinical trial of T2Candida, its flagship product for identifying five species of *Candida*.

Distinguishing rapidly between similar organisms, one of which can have a problematic antibiotic resistance, has important infection control implications. Dr. Ginocchio highlighted these pairs:

- *Klebsiella pneumoniae* (KPC) versus *E. coli*.
- *Acinetobacter* spp (MDRO) versus *E. coli*.
- *S. aureus* (MRSA) versus coagulase-negative staphylococci.
- *E. faecalis* (VRE) versus *E. faecium*.
- *S. pneumoniae* and *S. bovis* versus *S. viridans*.
- *C. krusei* versus *C. albicans*.

In closing, she reminded her ASM audience that rapid detection means nothing without rapid transmission of results, and she pointed to the results of a study in which one medical center instituted PNA FISH for coagulase-negative staphylococci but didn’t put in place an active notification program. The authors wrote, “Without active notification or antimicrobial stewardship intervention, a pre- and postimpact analysis showed no benefit of this assay with respect to the length of hospital stay or vancomycin use” (Holtzman C, et al. *J Clin Microbiol*.

2011;49:1581–1582).

“It’s not necessarily the laboratory’s issue,” Dr. Ginocchio says. “We can send the information to the doctor’s or the nurse’s phone, but if no one is there to receive and act on the information in a timely fashion, the result goes for naught.” There must be a highly collaborative interaction between the laboratory, pharmacy, and medical staff. To benefit from the use of rapid assays, all pieces must be in place.

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