Volume 3, Issue 1 Winter 2018

CLSI

CLSI Subcommittee on Antimicrobial Susceptibility Testing

CLSI AST News Update

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The CLSI **Outreach Working Group (ORWG)** is providing this Newsletter to highlight some recent issues related to antimicrobial susceptibility testing (AST) and reporting. We are listing links to some new educational materials and reminding you where you can find information about the CLSI AST Subcommittee proceedings.

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CLSI 2018 AST Webinar: M100, M02, and M07 Updates

This hour and a half webinar will help you identify the latest changes in the updated editions of M100, M02, and M07. Don't miss the opportunity to learn directly from leading AST experts.

Date Options:

February 7, 2018 | 1:00-2:30 PM Eastern (US) Time
February 8, 2018 | 3:00-4:30 PM Eastern (US) Time

Moderator: Janet A. Hindler, MCLS MT(ASCP) F(AAM)
Speakers: Romney M. Humphries, PhD, D(ABMM)
Audrey Schuetz, MD, MPH, FCAP

Nonmember Price: $99.00. | Earn 1.5 PACE® CE credits.
Member discounts apply.

Register today.

What does the CLSI AST Subcommittee do?

The first edition of the CLSI AST News Update (Volume 1, Issue 1, Spring 2016) described details about the organization and operation of the CLSI AST Subcommittee.

- Access that newsletter **here.**
- To learn more about upcoming or past meetings, click **here.**
- CLSI posts meeting minutes and summaries for public access **here.**

Interested in becoming a CLSI volunteer? Learn more here.

Please remember that the CLSI AST Subcommittee welcomes suggestions from you about any aspect of CLSI documents, educational materials, or this Newsletter.

CLSI AST Subcommittee Partnerships

Representatives with expertise in antimicrobials from the following organizations attend and participate in CLSI AST Subcommittee meetings and aid in dissemination of information regarding CLSI decisions and AST issues.

American College of Clinical Pharmacy Infectious Diseases Practice and Research Network (ACCP INFD PRN)

American Society for Microbiology (ASM)

Association of Public Health Laboratories (APHL)

ASTM International

College of American Pathologists (CAP)

European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Infectious Diseases Society of America (IDSA)

Pediatric Infectious Diseases Society (PIDS)

Society for Healthcare Epidemiology of America (SHEA)

Society of Infectious Disease Pharmacists (SIDP)

Susceptibility Testing Manufacturers Association (STMA)

Updated CLSI AST Documents Are Here!
So what's new?

Nomenclature changes:
Propionibacterium acnes to *Cutibacterium acnes*
Clostridium difficile to *Clostridioides difficile*
Enterobacter aerogenes to *Klebsiella aerogenes*

M100 28th Edition

New Breakpoints

Ceftazidime-avibactam for *Enterobacteriaceae* and *Pseudomonas aeruginosa*
Ceftolozane-tazobactam disk for *Enterobacteriaceae*
Dalbavancin for *Enterococcus* spp., *Streptococcus aureus*, *Streptococcus*, β-hemolytic group, *Streptococcus*, Viridans group.

New Recommendations:

Staphylococcus schleiferi
eCIM test for metallo-β-lactamases in *Enterobacteriaceae*

Updated Recommendations:

mCIM test for *P. aeruginosa*
Updated antibiogram table for anaerobes
(delete Modified Hodge Test)

Reformatting:

All ECVs moved to ECV Appendix G
Separate QC tables for β-lactam combination agents

M02 13th Edition and M07 11th Edition

Updated content throughout to match M100
Reformatted several sections for user clarity

New recommendations:

Testing *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi*

Updated recommendations:

Summary of drugs within drug classes
Explanation of gram-negative β-lactamases
"Growth method" of inoculum preparation now "broth culture method"
Maintenance and subculture of QC strains

Added "visuals":

Disk Diffusion Reading Guide (M02)
Growth control and skipped wells (M07)

Archive of Retired Breakpoints

An archive of breakpoints removed from M100 since 2010 together with the rationale for their removal is available **here.**

Similarly, an archive of methods removed from M100 since 2017 is available **here.**

Webinars

Archived on-demand webinars are available free of charge six months after the scheduled event for CLSI members.

On-Demand Webinars:

- *Practical Recommendations for Antifungal Susceptibility Testing and Reporting in Clinical Laboratories: New Drugs, New Breakpoints, New Guidelines (Fall 2016)*
- *Facts and Fiction about Colistin from Clinical and Public Health Perspectives (Fall 2016)*
- *Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems (Summer 2016)*

Learn more about on-demand webinar availability **here.**

Upcoming Webinars:

CLSI 2018 AST Webinar: M100, M02, and M07 Updates

February 7, 2018 | 1:00-2:30 PM Eastern (US) Time
or
February 8, 2018 | 3:00-4:30 PM Eastern (US) Time

Current Recommendations for Antimicrobial Susceptibility Testing of Enterococcus spp.

March 14, 2018 | 1:00-2:00 PM Eastern (US) Time
Presenter:

Stella Antonara, PhD D(ABMM)
Assistant Director, Clinical Microbiology and Immunoserology
Department of Pathology and Laboratory Medicine
Nationwide Children's Hospital, Columbus, OH

Learn more about these webinars on page 1 of this newsletter or **register here.**

ASM/CLSI 2017 AST Webinar Series

ASM and CLSI have recently completed a webinar series entitled "A Comprehensive Course in Antimicrobial Susceptibility Testing" which is geared towards bench level technologists. **The 14 programs are now available on demand here.**

Part I. Fundamentals of Susceptibility Testing, Reporting, and Test Validation

Introduction to Antimicrobials
Understanding Manual Antimicrobial Susceptibility Tests and Their Enduring Value
Introduction to Automated Antimicrobial Susceptibility Testing
Antimicrobial Results Reporting: Communicating Effectively with Clinicians
Quality Control and Validation/Verification of Antimicrobial Susceptibility Tests (ASTs)

Part II. Mechanisms of Resistance, Antimicrobial Stewardship, and Infection Prevention

Gram-Positive Resistance Mechanisms and Testing: Staphylococcus
Gram-Positive Resistance Mechanisms and Testing: Enterococcus/Streptococcus
Gram-Negative Resistance Mechanisms and Testing: Enterobacteriaceae
Gram-Negative Resistance Mechanisms and Testing: Non-Enterobacteriaceae
Infection Prevention and Stewardship: Implications Beyond the Laboratory

Part III. Special Antimicrobial Susceptibility Tests

Susceptibility Testing for Fastidious Bacteria, Including Anaerobes
Susceptibility Testing for Yeasts and Filamentous Fungi
Susceptibility Testing for Slow and Rapid Growing Mycobacteria
Answering Today's Most Commonly Encountered AST Questions: A Case Based Approach

Check It Out! Educational Workshops Held at CLSI meetings

Nicole Scangarella-Oman

To coincide with the January and June CLSI Committee Weeks, the ORWG coordinates a live Educational Workshop, typically held on the Saturday evening prior to the start of the AST Subcommittee Working Group meetings.

The June 2017 workshop, held in Philadelphia, was "New and Successful Approaches to Antimicrobial Stewardship: The Role of the Microbiology Laboratory." Clinical microbiology laboratories play a critical role in implementing, influencing, and executing successful antimicrobial stewardship programs with the ultimate goal of improving patient care. Some of the topics highlighted during this workshop included: antimicrobial stewardship in the hospital, long-term care and outpatient settings, the role of rapid diagnostics and interpretive reporting, and the role of antibiograms in antimicrobial stewardship.

The next workshop, "Epidemiological Cutoff Values (ECVs): Their Development and Use," will be held on Saturday, January 27, 2018 in Dallas, Texas.

PowerPoint presentations from past workshops can be found **here.**

Future CLSI AST Meetings!

January 25-30, 2018
Dallas, Texas, USA

May 31-June 5, 2018
San Diego, California, USA

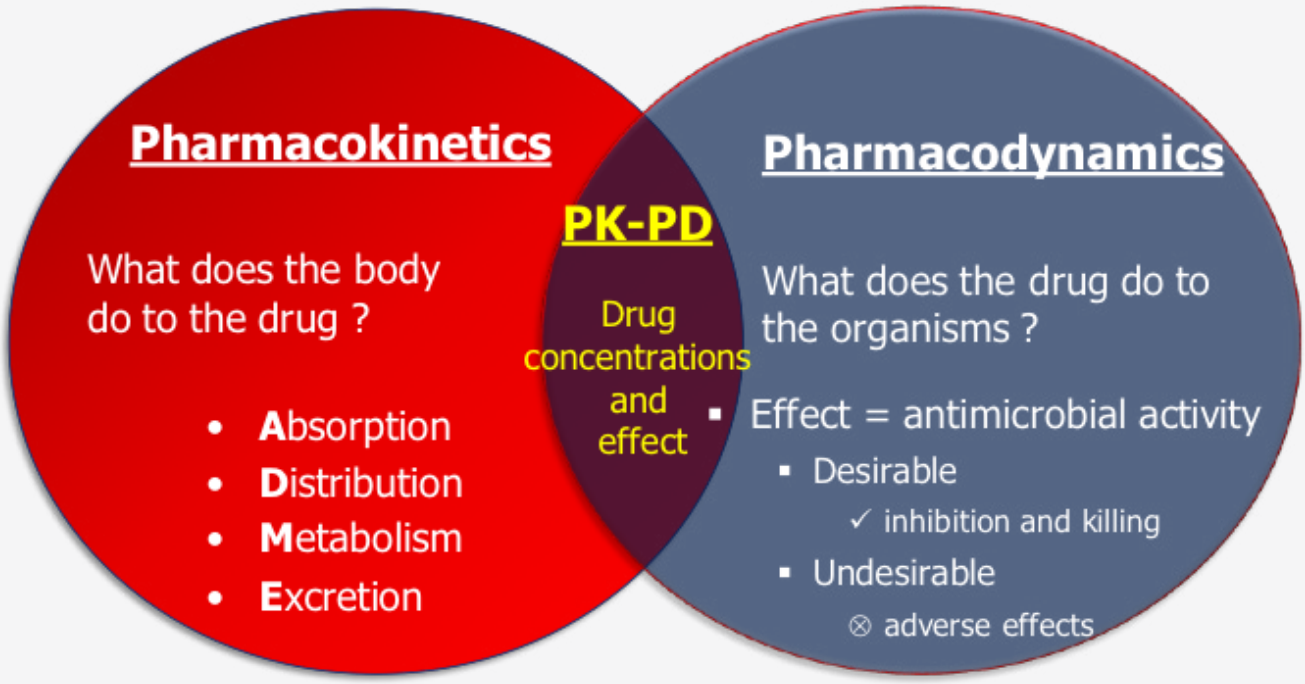
The 21st Century Cures Act – Exciting News

In the last issue of this **Newsletter,** we explained details of the 21st Century Cures Act. As part of meeting the provisions of 21st Century Cures Act, on December 13, 2017, the FDA launched the **Antimicrobial Susceptibility Test Interpretive Criteria website** which includes recognition of many CLSI disk diffusion and MIC interpretive criteria (also known as breakpoints). This is a major accomplishment for CLSI and FDA. Individuals from both groups have worked diligently to accomplish this goal and we applaud them for their efforts! We urge you to review the website to learn about this development which will be explained a bit more during our Annual Update Webinar in February.

Understanding Pharmacokinetics (PK) and Pharmacodynamics (PD)

Patricia J. Simner, Johns Hopkins Medicine and Linda Miller, CMID Pharma Consulting

Figure 1: Interplay between Pharmacokinetics and Pharmacodynamics of Antimicrobial Agents



As microbiologists performing antimicrobial susceptibility testing (AST), we may have heard of the terms pharmacokinetics (PK) and pharmacodynamics (PD). PK and PD parameters of antimicrobials are used to optimize dosing of antimicrobials to maximize their effectiveness while minimizing toxicity to patients **(see Figure 1).** PK/PD is also critical in the process used to determine breakpoints, which are the criteria applied to the Minimum Inhibitory Concentration (MIC) of a patient isolate. Breakpoints are used to categorize the MIC of an isolate as "Susceptible," "Intermediate," "Susceptible-Dose-Dependent", "Non-Susceptible," or "Resistant." When the Clinical and Laboratory Standards Institute (CLSI) sets breakpoints, they use the following different cut-offs:

- MICs of wild-type (WT) isolates (in general, these are isolates lacking resistance mechanisms to the drug) that provide an **epidemiologic cutoff value (ECV)**
- Animal or *in vitro* PK/PD models that provide a **non-clinical PK/PD cutoff**
- PK/PD clinical exposure response (CER) data from patients in clinical trials that provide a **CER cutoff**
- Success/failure data by minimal inhibitory concentration (MIC) from clinical trial data that provide a **clinical cutoff**

These four cutoff values are then used to develop a breakpoint that clinical microbiologists use with MICs to provide susceptibility reports to clinicians. The breakpoint values are those that are published in M100, Performance Standards for Antimicrobial Susceptibility Testing for interpretation of MIC values based on the drug-organism combination. The objective of this article is to provide a basic overview of PK and PD and what they mean to the laboratorian performing AST.

What does the lab need to know about Pharmacokinetics?

PK answers the question *"What does the body do to the drug?"* PK studies evaluate drug absorption, distribution, metabolism and excretion from the body. These parameters are usually measured by studying the achievable drug levels in blood and other body fluids (eg, CSF) in healthy volunteers. Most antimicrobial agents are protein-bound, ranging anywhere from 30% to 95% depending on the agent. While PK can be measured as total drug concentration, it is only the unbound (free) drug that has activity against bacterial pathogens. Therefore, unbound (free) drug concentrations are generally used in assessment of PK for setting breakpoints or determining a dose.

Understanding Pharmacokinetics (PK) and Pharmacodynamics (PD) (*Continued*)

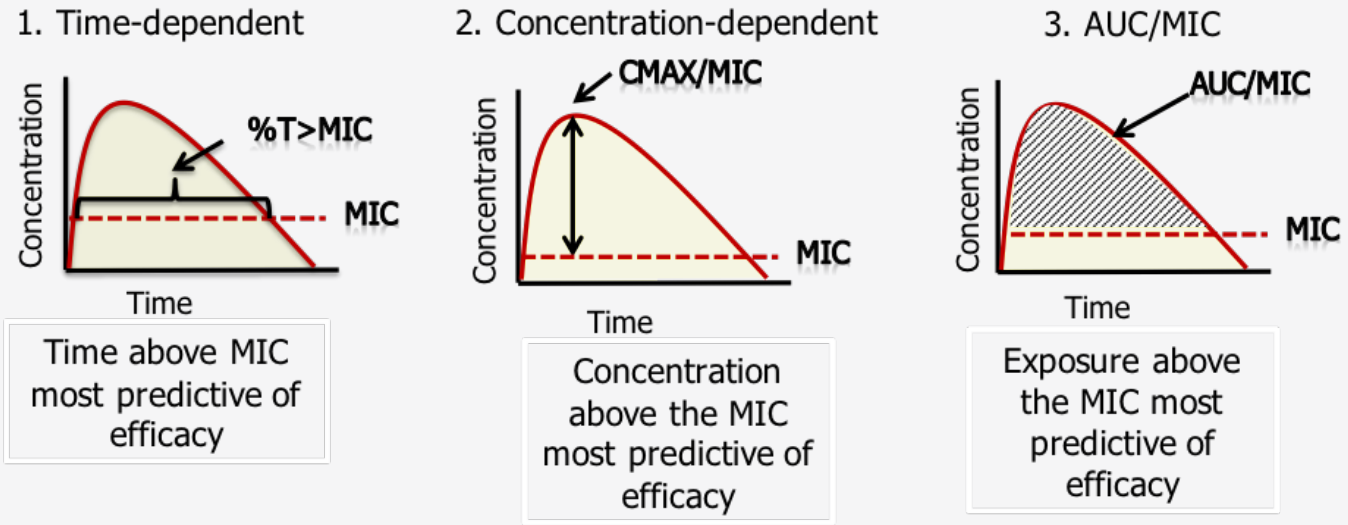
What about Pharmacodynamics?

PD, on the other hand, studies the relationship between unbound drug concentration over time and the resulting antimicrobial effect on the organism. PD answers the question *"What does the drug do to the organism?"* Ideally, the effect of an antimicrobial agent is to eradicate the infecting organism without adverse effects to the patient.

Antimicrobial agents are generally classified into three classes based on *in vitro* PD drug effect: 1) time-dependent, 2) concentration-dependent, or 3) area under the curve (AUC)/MIC ratio **(see Figure 2):**

1. Time-dependent bactericidal effect:
   - Antimicrobials classified as time-dependent require that the drug concentrations be above the MIC for a certain percentage of the dosing interval to effectively kill the organism.
   - Generally, once the target "time above MIC" is reached for a particular isolate, increasing the free drug concentrations of these drugs above the standard treatment dose has no further effect on bacterial killing of that isolate.
   - Examples of time-dependent antimicrobials are penicillins, cephalosporins, carbapenems, and aztreonam.
2. Concentration-dependent bactericidal effect:
   - Concentration-dependent antimicrobials achieve increasing bactericidal effect with increased serum levels of the drug.
   - These drugs are dosed to achieve maximum safe concentrations at the infection site for optimal bactericidal activity (eg, concentrations that are 10 times the MIC for aminoglycosides).
   - Examples of concentration-dependent antimicrobials are aminoglycosides, daptomycin.

Figure 2: Pharmacodynamic Classification of Antimicrobial Agents.



In the three graphs, "time" refers to the dosing interval. "Concentration" refers to the amount of drug attained over time in a patient's blood following administration of the drug. A dose of antimicrobial is initially administered at time 0. The concentration increases, then decreases and at a certain time, a subsequent dose may be given.

Abbreviations: %T > MIC, length of time the concentration of drug in the patient's serum remains above the MIC; CMAX, highest (maximum) concentration of drug attained during the dosing interval; AUC, area under the curve calculated by examining the length of time the drug concentration remains above the MIC together with the overall drug concentration achieved over this time frame. The broken horizontal MIC line refers to the susceptible breakpoint for the antimicrobial drug-organism combination.

Understanding Pharmacokinetics (PK) and Pharmacodynamics (PD) (*Continued*)

3. Area under the curve (AUC) /MIC ratio:

- Efficacy of antimicrobials in this group is dependent on the total concentration of the drug achieved over 24 hours (eg, area under the curve [AUC]0-24) above the MIC of the organism.
- Examples of AUC/MIC antimicrobials are fluoroquinolones, vancomycin.

So how are PK and PD used in the determination of the breakpoint?

The MIC of a drug for an organism is compared to the achievable unbound drug concentrations at a site of infection, most commonly in blood. Animal or *in vitro* models of infection are used to identify the PK/PD parameter and magnitude of that parameter (ie, % T >MIC, cMAX/MIC, or AUC/MIC [see Figure 2]) that best correlates with efficacy. Generally, the "susceptible" breakpoint is set at the highest MIC where the PK/PD target for efficacy is achieved in approximately 90% of the patient population using standard dosing. As described above, other information, including the epidemiologic cutoff value, the CER cutoff and the clinical cutoff are also used to determine the breakpoint for a drug-organism combination.

Tying It All Together – An Example to Illustrate How CLSI Used PK and PD Data to Establish Ceftazidime Breakpoints for *Enterobacteriaceae*

Now that we understand what PK and PD mean, let's review by looking at an example—the establishment of ceftazidime breakpoints for the *Enterobacteriaceae*. When determining breakpoints, population pharmacokinetics and Monte Carlo simulations are utilized along with the PK/PD targets that have correlated with efficacy in models or in clinical trials. Monte Carlo simulation is a statistical tool that can use a limited data set to predict the likelihood of PK/PD target attainment for a population of patients. In general, the goal is to achieve at least 90% target attainment. The human PK of an antimicrobial varies by individual (ie, absorption, distribution, metabolism, and excretion of the drug in the body over time). Monte Carlo simulations are used to incorporate the potential variability expected in the patient population for an antibiotic and to simulate the likelihood of target attainment for efficacy at different MICs.

Figure 3: Percent probability of PK/PD target attainment for ceftazidime.

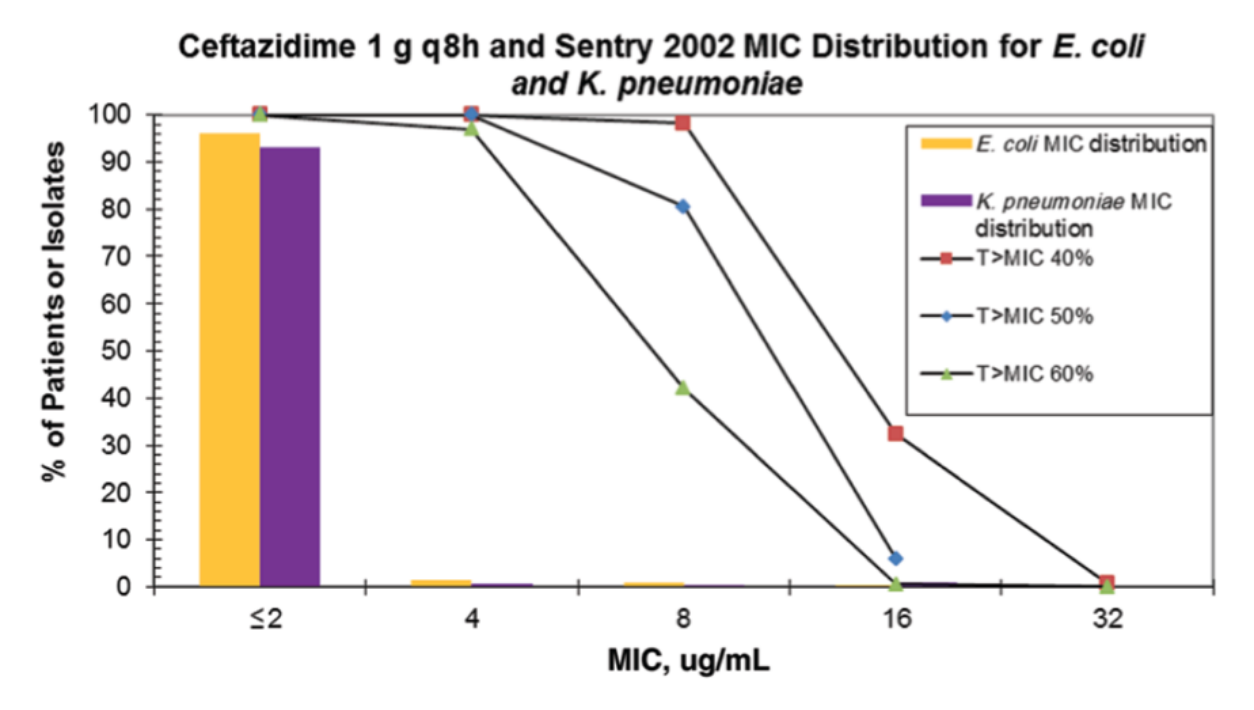


Figure 3 is derived from Monte Carlo simulation modeling for ceftazidime plotted against the MIC distributions for *Escherichia coli* and *Klebsiella pneumoniae*. These data were used by CLSI to help define the clinical breakpoints for ceftazidime. The figure demonstrates that when ceftazidime is dosed intravenously at 1 g **every 8 hours,** target attainment rates (black lines on figure) of > 90% are achieved up to an MIC of 4 µg/mL for T > MIC targets of 40%, 50%, and 60%. At an MIC of 8 µg/mL, only the lower threshold T > MIC target of 40%

Understanding Pharmacokinetics (PK) and Pharmacodynamics (PD) (*Continued*)

allows a 90% target attainment rate. Animal models indicate that for cephalosporins and *Enterobacteriaceae* a time above MIC of 50% was consistently needed for efficacy. Therefore, for ceftazidime dosed intravenously at 1 g **every 8 hours,** the highest MIC for which at least 90% of patients would be expected to meet the 50% T > MIC target would be an MIC of 4 µg/mL. Thus, the non-clinical PK/PD cut-off is 4 µg/mL. As described earlier in this article, CLSI also evaluates other available data (eg, the epidemiologic cut-off, clinical cut-off, and clinical exposure response cut-off) to set a breakpoint. After evaluation of all the relevant data, CLSI set the susceptible breakpoint for ceftazidime and *Enterobacteriaceae* at ≤ 4 µg/mL.

Recommended Reading:

Ambrose PG, Bhavnani SM, Rubino CM, Louie A, Gumbo T, Forrest A, et. Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. *Clin Infect Dis.* 2007;44(4):79-86.

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Quintiliani R. Using pharmacodynamic and pharmacokinetic concepts to optimize treatment of infectious diseases. *Infect Med.* 2004;21:219-233.

Turnidge JD. Susceptibility Test Methods: General Considerations. In: Jorgensen JH and Pfaller MA eds. *Manual of Clinical Microbiology.* 11th Edition. Washington, DC: ASM Press, 2015:1246-1252.

Case Study
Direct Detection of MRSA/MSSA From Positive Blood Cultures

April Abbott and Jennifer Dien Bard, Children's Hospital, Los Angeles

A 14-year-old female was brought to the Emergency Department with vomiting and fever. One week prior, the patient had been seen at an outpatient clinic and diagnosed with a viral respiratory illness that had gotten progressively worse. At presentation, physicians were concerned that the patient may have bacterial pneumonia and sepsis; therefore, blood and sputum cultures were obtained. After twelve hours of incubation, the first blood culture became positive and gram-positive cocci in clusters were observed on Gram stain. Per laboratory protocol, a multiplex molecular assay was performed directly from the positive blood culture bottle to provide early identification and antimicrobial susceptibility information. Results from the molecular test are shown in the preliminary report in Figure 1.

Figure 1: Initial Workup Directly From Positive Blood Culture Bottle.

Blood Culture
Obtained: 1/2/18 10:30 am
Received: 1/2/18 11:45 am

1/3/18 6:15 am
Preliminary Report:
Gram-positive cocci in clusters

1/3/18 9:00 am
Preliminary Report based on molecular test:
Methicillin-resistant *Staphylococcus aureus*
Further susceptibility results to follow

Molecular assay result:

Staphylococcus	Detected
Staphylococcus epidermidis	Not Detected
Staphylococcus aureus	Detected
Staphylococcus lugdunensis	Not Detected
Streptococcus	Not Detected
Streptococcus agalactiae	Not Detected
Streptococcus pneumoniae	Not Detected
Streptococcus pyogenes	Not Detected
Enterococcus faecalis	Not Detected
Enterococcus faecium	Not Detected
mecA	Detected
vanA/B	Not Detected

Figure 2: Confirmatory Workup from Solid Media.

1/4/18 7:18 am
Amended (Preliminary) Report:
1. Methicillin-resistant *Staphylococcus aureus*.
   Further susceptibility results to follow
2. *Staphylococcus haemolyticus*.
   Probable contaminant.

1/6/18 5:10 am
Amended (Final) Report:
1. Methicillin-susceptible *Staphylococcus aureus*.
2. *Staphylococcus haemolyticus*, methicillin-resistant.
   Probable contaminant.

1. *Staphylococcus aureus*

	MIC (µg/ml)	
Clindamycin	≤0.5	S
Daptomycin	≤0.5	S
Linezolid	≤0.5	S
Oxacillin	≤2	S
Trimeth-sulfa	≤1/20	S
Vancomycin	≤1	S

2. *Staphylococcus haemolyticus*

	MIC (µg/ml)	
Clindamycin	>4	R
Daptomycin	≤0.5	S
Linezolid	≤0.5	S
Oxacillin	>4	R
Trimeth-sulfa	≤1/20	S
Vancomycin	≤1	S

Case Study
Direct Detection of MRSA/MSSA From Positive Blood Cultures (*Continued*)

Results from the molecular test **(see Figure 1)** indicated the presence of *Staphylococcus aureus, Staphylococcus* spp., and *mecA* gene. Given that *mecA* was detected, the laboratory reported the result as methicillin-resistant *Staphylococcus aureus* (MRSA). The following day, growth on solid media revealed two colony types that were identified by MALDI TOF MS as *S. aureus* and *S. haemolyticus*. Preliminary report was amended to include the coagulase-negative staphylococci (*S. haemolyticus*). Antimicrobial susceptibility testing (AST) by a commercial system was performed on the *S. aureus* isolate. About 18 hours later, the AST result of the *S. aureus* isolate revealed oxacillin minimum inhibitory concentration (MIC) of ≤ 2 µg/ml (S). Cefoxitin screen by disk diffusion confirmed the *S. aureus* isolate to be methicillin-susceptible. The isolate was also confirmed to be *S. aureus* by slide coagulase test. AST of the *S. haemolyticus* revealed oxacillin MIC of > 4 µg/ml (R). Report was again amended to reflect that the culture was growing a methicillin-susceptible *S. aureus* (MSSA) and a methicillin-resistant *S. haemolyticus* **(see Figure 2).** The physician was notified of the amended report and therapy was narrowed from vancomycin to cefazolin since antimicrobial coverage against solely the *S. aureus* was needed. The microbiology director requested that an investigation be conducted to determine how the error occurred. Results of the investigation are presented below.

The performances of blood culture molecular multiplex assays have high concordance compared to culture, especially in cases of monomicrobial infections.[1-3] In contrast, erroneous results and lower concordance are reported when the positive blood culture is polymicrobic.[1-3] The biggest limitations of such multiplex assays run on polymicrobial blood cultures is that the organism with the higher bacterial load may dominate and prevent the other target(s) from being detected, or one target may be present below the limit of detection of the assay. In the case described here, the discrepancy occurred because the blood culture was thought to be monomicrobial and the *mecA* was assumed to be expressed in the *S. aureus* isolate when it was actually expressed in the *S. haemolyticus* isolate. This is due to the fact that in the presence of *S. aureus,* not only would the *"Staphylococcus aureus"* target be detected, but the *"Staphylococcus"* target would also be detected. Hence, the results can be interpreted as a lone *S. aureus* or a mixture of *S. aureus* and a separate *Staphylococcus* sp. This also applies to the *S. epidermidis* and *S. lugdunensis* targets. Another reason for the discrepancy (if there had not been a mixture of staphylococcal species in the sample) could be the presence of an altered staphylococcal cassette chromosome resulting in the so-called "drop-out phenomenon" which would result in detection of *mecA* despite phenotypic susceptibility. In this case, if discrepancy analysis does not yield a resolution, then MRSA would have been reported as final.

If the case were reversed and *mecA* not detected from a blood culture grew MRSA and another *Staphylococcus* sp., one may reason that the false-negative report of the *mecA* gene may be due to high target expression of the two isolates.

S. aureus, both as a cause of sepsis and superinfection following a viral respiratory infection, primarily influenza, has a high mortality rate; therefore, rapid differentiation of MRSA from MSSA for appropriate treatment is critical for patient care. Laboratories must be aware of the limitations of any assay performed and possess the ability to quickly resolve testing issues, specifically when they affect antimicrobial therapy.

Best Practice Pearls:

- Phenotypic susceptibility testing is required to confirm the detection or absence of resistance genes from molecular assays performed on positive blood cultures.
- Identification of the isolate(s) must be confirmed in cases of discrepant susceptibility results.
- If discrepancy remains unresolved in a case such as this, report as MRSA.

CLSI provides a table **here** to assist laboratorians in investigating discrepant susceptibility results and guide them on how to report final results when there is discordance between molecular and phenotypic assays for MRSA. In cases where the presence or absence of *mecA* detection in *S. aureus* contradicts the cefoxitin and/or oxacillin result, identification and susceptibility should be repeated and bacterial growth on agar plates should be carefully screened to rule out mixed culture. Another option would be to perform an additional molecular test to screen for *mecA* from isolated colonies. If the discrepancy is not resolved, it is recommended that the isolate be reported as MRSA to ensure appropriate antimicrobial coverage. In the case presented here, the discrepancy was resolved by confirming methicillin resistance in the *S. haemolyticus* and hence the preliminary MRSA report was amended to MSSA. The strategy for handling discrepancies in this case also apply when screening for vancomycin resistance by detection of *vanA/B* gene in *Enterococcus* species.

Case Study
Direct Detection of MRSA/MSSA From Positive Blood Cultures (*Continued*)

References

1 Altun O, Almuhayawi M, Ullberg M, Ozenci V. Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. *J. Clin Microbiol.* 2013;51(12):4130-4136.

2 Mestas J, Polanco CM, Felsenstein S, Dien Bard J. Performance of the Verigene Gram-positive blood culture assay for direct detection of Gram-positive organisms and resistance markers in a pediatric hospital. *J Clin Microbiol.* 2014;52(1):283-287.

3 Martinez RM, Bauerle ER, Fang FC, Butler-Wu SM. Evaluation of three rapid diagnostic methods for direct identification of microorganisms in positive blood cultures. *J Clin Microbiol.* 2014;52(7):2521-2529.

Burning Question - When Should Clinical Microbiology Laboratories Perform Carbapenemase Detection Tests?

Lars Westblade

Carbapenem resistance is one of the most concerning forms of antimicrobial resistance, particularly when encountered in the *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.[1,2] Organisms displaying overt resistance to carbapenems can be divided into two groups: 1) carbapenemase-producing organisms (CPO) that express carbapenemases, enzymes that hydrolyze the carbapenem β-lactam ring, and 2) non-carbapenemase-producing-carbapenem-resistant organisms (non-CP-CRO) that have reduced susceptibility to carbapenems due to expression of cephalosporinases (ESBL and AmpC) coupled with cell permeability defects.[1,2] Unlike non-CP-CRO, the genes associated with CPO are readily transferrable to many gram-negative species as these are often located on mobile genetic elements (eg, plasmids), increasing the potential for widescale spread.[1,2]

Carbapenemases belong to one of three classes based upon their amino acid sequence: Ambler class A, B, or D. Class A (eg, KPC) and D (eg, OXA-48-type) enzymes possess a serine-based hydrolytic mechanism, while class B carbapenemases (eg, NDM, IMP, VIM) are metallo-β-lactamases and require one or two zinc ions for catalytic activity.[3] KPC is endemic in the United States, Israel, South America and some countries in Europe and Asia, while OXA-48-type and NDM enzymes predominate in North Africa/Europe and Asia, respectively.[1,2] However, as a result of widespread international travel and exposure to medical care, the association between a specific resistance mechanism and a given region or country is not definite and may change.[1]

There are many phenotypic and genotypic carbapenemase detection tests (CDT) available for use in clinical laboratories **(see Table 1).**[1,2,4] Typically, phenotypic assays detect carbapenemase activity in bacterial isolates recovered in culture, while genotypic assays permit detection of carbapenemase genes directly in clinical specimens (eg, positive blood cultures or rectal swabs) or from organisms isolated in culture. Differentiation between non-CP-CRO and CPO is not recommended by the Clinical and Laboratory Standards Institute (CLSI) for routine patient care, except for those laboratories that have not yet implemented the current CLSI *Enterobacteriaceae* carbapenem breakpoints.[5] ***Therefore, why should Clinical Microbiologists consider CDT when confronted with the increasing challenges facing laboratories today? Reduced operational costs, lack of test charge reimbursement, and a shortage of individuals entering the profession, to list but a few.***

Table 1. Selection of Phenotypic and Genotypic CDT Currently Available (modified from [1,2,4]).

Test (Manufacturer)	Method	Specimen Type	Turnaround Time (time to results from setting up the assay)	Carbapenemase Gene(s) Detected	Regulatory Status
Phenotypic CDT					
Carba NP	Color indicator of imipenem hydrolysis	Isolates of *Enterobacteriaceae* or *P. aeruginosa*	Same day	Not applicable (N/A)	Commercial version United States Food and Drug Administration (FDA) cleared
mCIM	Growth of carbapenem susceptible indicator strain around meropenem disk incubated with a CPO test strain	Isolates of *Enterobacteriaceae* or *P. aeruginosa*	Next day	N/A	Laboratory Developed Test (LDT)
eCIM	Growth of carbapenem susceptible indicator strain around meropenem disk incubated with a CPO test strain in the presence and absence of EDTA	Isolates of *Enterobacteriaceae* (modification of mCIM that allows differentiation between serine- and metal-dependent carbapenemases)	Next day	N/A	LDT

Burning Question - When Should Clinical Microbiology Laboratories Perform Carbapenemase Detection Tests?(*Continued*)

Table 1. Selection of Phenotypic and Genotypic CDT Currently Available (modified from [1,2,4]). (*Continued*)

Test (Manufacturer)	Method	Specimen Type	Turnaround Time (time to results from setting up the assay)	Carbapenemase Gene(s) Detected	Regulatory Status
Phenotypic CDT					
MALDI-TOF MS	Detection of carbapenem degradation products	Bacterial isolates	Same day	N/A	LDT
Genotypic CDT					
FilmArray® Blood culture identification panel (BioFire Diagnostics)	PCR	Positive blood culture broth with GNR	Same day	[a]bla_{KPC}	FDA cleared
Verigene® gram-negative blood culture test (Luminex Corporation)	Microarray	Positive blood culture broth with GNR	Same day	[b]bla_{IMP} bla_{KPC} bla_{NDM} bla_{OXA-48} bla_{VIM}	FDA cleared
GeneXpert® Carba-R (Cepheid)	PCR	Rectal swabs, isolates of *Enterobacteriaceae*, *P. aeruginosa*, *A. baumannii*	Same day	[c]bla_{IMP} bla_{KPC} bla_{NDM} bla_{OXA-48} bla_{VIM}	FDA cleared

Abbreviations: CPO, carbapenemase-producing organism; EDTA, ethylenediaminetetraacetic acid; GNR, gram-negative rods; LDT, laboratory developed test; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

[a]KPC resistance gene only reported when one of the following organisms is detected: *A. baumannii*, *Enterobacter cloacae* complex, *Escherichia coli*, *Klebsiella pneumoniae, Klebsiella oxytoca, Proteus* species, *P. aeruginosa, Serratia marcescens*.

[b]Carbapenemase resistance genes only reported when one of the following organisms is detected: *Acinetobacter* species, *Citrobacter* species, *Enterobacter* species, *E. coli, K. pneumoniae, K. oxytoca, P. aeruginosa, Proteus* species, *S. marcescens*.

[c]The Carba-R system does not perform organism identification, only molecular detection of carbapenemase genes.

First, as mentioned above, **if clinical laboratories have not implemented the current CLSI carbapenem breakpoints for *Enterobacteriaceae*** a CDT should be performed when isolates of *Enterobacteriaceae* exhibit a minimum inhibitory concentration (MIC) value of 2 µg/mL for ertapenem or 2-4 µg/mL for imipenem or meropenem.[5] However, laboratories should ardently strive to use current breakpoints for accurate identification of carbapenem resistance.

Second, controlling the spread of CPO, in particular carbapenemase-producing-carbapenem-resistant *Enterobacteriaceae* (CP-CRE), within institutions is critical. However, this is challenging because many CPO-infected patients are initially identified by routine antimicrobial susceptibility testing (AST), which may take up to five days to report. And CPO from diagnostic cultures represent the "tip of the iceberg" of patients harboring CPO. Therefore, some institutions have initiated surveillance for CPO (especially within their immunosuppressed patient populations) ranging from culture with selective and differential media with or without a CDT to molecular methods. Rapid CDT that screen for CPO colonization offer the opportunity to promptly implement infection control interventions resulting in reduced CPO transmission as demonstrated in practice.[2] Similarly, rapid CDT that detect and differentiate carbapenemases permit the identification of related cases during an outbreak.

Finally, infections with carbapenem-resistant organisms, especially bloodstream infections, remain difficult to treat and are associated with unacceptably high mortality rates.[1,2] Implementation of diagnostics that rapidly identify these organisms from positive blood cultures could improve patient outcomes by permitting earlier consultation with infectious diseases experts and prompt administration of effective empiric therapy. Indeed, consultation with infectious diseases specialists is linked to favorable outcomes for patients with *Staphylococcus aureus* bloodstream infections[6] and will likely benefit patients with invasive infections because of

Burning Question - When Should Clinical Microbiology Laboratories Perform Carbapenemase Detection Tests?(*Continued*)

CPO. Importantly, newer antimicrobials active against CPO often depend on the carbapenemase type, and this knowledge prior to conventional AST results could support decisions about use of these agents for empiric therapy. For example, most KPC and some OXA-48-type producing isolates are susceptible to ceftazidime-avibactam, but this drug has no activity against isolates that produce metallo-β-lactamases.[1,2]

In summary, an institution's local CPO prevalence and patient population will largely dictate the economic and clinical benefit of introducing CDT. Clinical Microbiologists should actively engage infectious diseases and infection control and prevention specialists and their antimicrobial stewardship programs to determine the necessity, method and frequency of such testing. However for the reasons presented above, clinical microbiology laboratories are strongly encouraged to adopt, or have readily available access to, some form of CDT that permits accurate detection of CPO in their institutions. In addition, as mentioned above, all laboratories are strongly encouraged to use current CLSI recommended carbapenem breakpoints.

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Respiratory Illnesses and the Need for Antibiotic Stewardship!

Angella Charnot-Katsikas

'Tis the Season! Good holiday cheer and kissing loved ones from far and near can bring along a heaping pile of respiratory illness. Along with this comes a generous serving of antibiotics. In fact, respiratory infections are the primary clinical indication for which antibiotics are prescribed and overused.[1] Studies show that up to 75% of patients with an acute upper respiratory tract infection are prescribed antibiotics; moreover, broad spectrum agents are selected > 60% of the time and up to two-thirds of these prescriptions may be inappropriate.[1,2] In November 2017, the world celebrated Antibiotic Awareness Week, to raise awareness of the importance of appropriate antibiotic prescribing and use. It would therefore seem obvious that a great place to start getting judicious about antibiotics would be somewhere in respiratory land.

Antibiotic stewardship for respiratory infections can be orchestrated in several ways, and the clinical microbiology laboratory can play a key role in these efforts. For example, the use of multiplexed panels that detect an array of respiratory pathogens is commonplace in many clinical labs. One would expect that antimicrobial stewardship would be enhanced by the rapid information these panels provide. The almost-immediate knowledge that a patient is ill with a virus such as influenza or RSV should imply he or she will not be treated for this infection with an antibiotic. This is sometimes true, but patients are still often prescribed and then maintained on antibiotics after rapid test results detect a viral cause of illness.[3] Some studies have shown the use of the rapid panels has led to a decreased duration of antibiotic use.[4,5] Additional benefits have included decreased rates of admission and length of hospitalization. However, findings to the contrary have also been reported. Shiley et al. found that antibiotics were discontinued for only 6 of 131 (4.6%) adult inpatients subsequently determined to have a viral respiratory illness; the authors attributed this, in part, to physician concern for bacterial co-infection.[3] This is not always unreasonable, as studies have shown rates of bacterial-viral co-infections can range from 3 to 82% in cases of community acquired pneumonia (CAP) requiring hospitalization. This range is wide and is partially attributed to variability in overall rates of pathogen detection.[6-8] Of course, it must be remembered that bacterial/viral co-infection rates may be lower in less severely ill patients who do not require hospitalization.

In a more recent study evaluating rapid diagnostics (results available within 2 hours), Gelfer et al. noted fewer days of antibiotic therapy when a viral pathogen was detected in concert with a low procalcitonin level; however, antibiotics were discontinued in only 4 of 18 (22%) hospitalized patients with viral infections.[9] In this study, the authors highlight the need for real-time communication with clinicians or an antimicrobial steward.

Table 1. shows approaches a laboratory can take to help curb overutilization of antibiotics for viral respiratory infections. Providing rapid testing is important, but it is equally important that results are rapidly communicated and acknowledged by clinicians, in order to meaningfully impact decision making and overall patient care. Further, laboratories should offer testing that can best accommodate the populations they serve, such that testing is not only rapid but appropriate. One way this can be accomplished is by offering multiplex panel tests as well as targeted tests, as appropriate. For example, large multiplex panel tests may be useful for immunocompromised patients and inpatients, particularly during times when the prevalence of circulating viruses, such as influenza, is low. On the other hand, outpatients may sometimes benefit from targeted testing when influenza is prevalent and circulating strains in the community are known. Detecting a viral cause of illness in either scenario may lead to avoidance of unnecessary antibiotics.

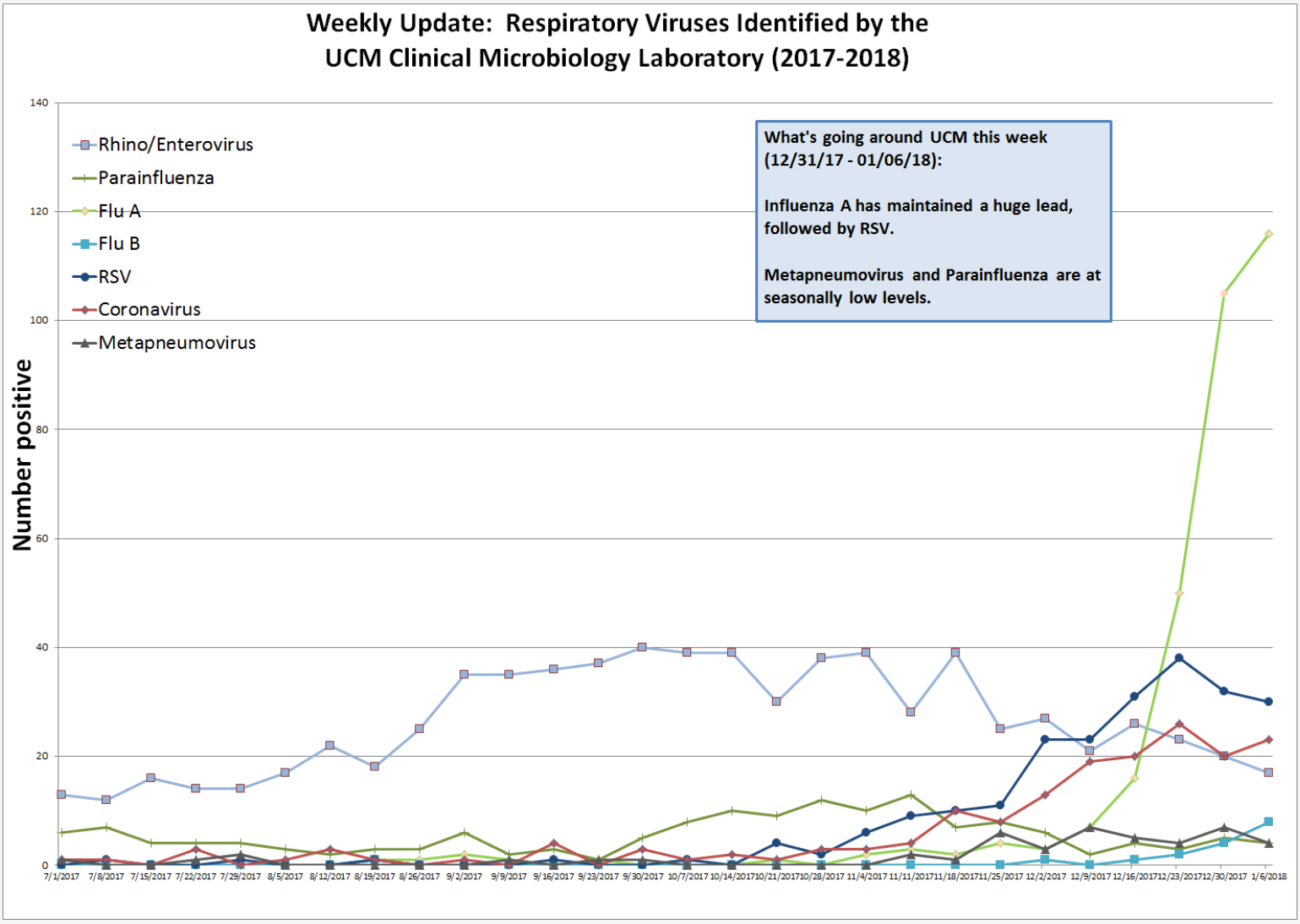
To this end, a laboratory can also share its respiratory pathogen findings with clinicians on a regular basis **(see Figure 1)**. This way, clinicians know which pathogens are circulating and prevalent in their given population, which can help them make more informed decisions regarding testing and management. Yet another approach is the implementation of justification-based antibiotic ordering. If clinicians are aware that they may have to provide justification for their use of antibiotics, they may think twice about prescribing antibiotics for a likely or documented viral infection. For example, clinicians may become more judicious when prescribing antibiotics for an uncomplicated upper respiratory infection such as pharyngitis when there is no evidence of a bacterial cause, if they have to justify this at the time of antibiotic ordering. In the end, multiple strategies and open lines of communication between the laboratory, clinicians, antimicrobial stewards, and patients are necessary to effect lasting change in improving antimicrobial stewardship.

Respiratory Illnesses and the Need for Antibiotic Stewardship! (*Continued*)

Table 1. Antimicrobial Stewardship Strategies for the Microbiology Laboratory Regarding Respiratory Pathogen Testing

Regularly update clinicians regarding respiratory pathogens identified in your institution and the community, as applicable.
Deliver results quickly using rapid and appropriate respiratory pathogen detection panels (multiplex and/or targeted).
Communicate respiratory pathogen results to clinicians in real-time (eg, via an antimicrobial steward or electronic direct reporting).
Participate in the development of Electronic Medical Record interventions to assist with stewardship efforts (eg, indication-based antimicrobial ordering).
Participate in audits and feedback regarding antimicrobial treatment of respiratory infections in your institution.

Figure 1: Respiratory Viruses Identified at a Clinical Microbiology Laboratory



UCM: The University of Chicago Medicine

Respiratory Illnesses and the Need for Antibiotic Stewardship! (*Continued*)

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AST of Bacteria Associated With Community-Acquired Pneumonia

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The majority of non-severe cases of community- acquired pneumonia (CAP) in the U.S. are caused by influenza viruses, *Staphylococcus pneumoniae,* and less commonly *Haemophilus influenzae, Moraxella catarrhalis, Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. Frequently used empirical therapy for outpatients includes doxycycline or a macrolide which will cover most bacterial pathogens causing CAP. For patients who are hospitalized, the combination of a broad-spectrum β-lactam (eg, ceftriaxone) plus a macrolide or doxycycline to cover for atypical organisms that are not treated by β-lactams is prescribed. Monotherapy with a respiratory fluoroquinolone (levofloxacin or moxifloxacin) is an alternative, particularly if the patient cannot receive β-lactam therapy. Blood culture and respiratory cultures are often performed for patients who are hospitalized with CAP. Susceptibility testing may be appropriate for *S. pneumoniae, H. influenzae,* and *M. catarrhalis,* if isolated. The CLSI document M100 28th edition addresses testing of *S. pneumoniae* and *H. influenzae,* whereas information regarding testing of *M. catarrhalis* is found in M45-A3.

Recent US data indicate that both penicillin and fluoroquinolones remain excellent treatment options for *S. pneumoniae*, provided the infection does not involve the central nervous system. Based on a recent large US survey, greater than 95% of isolates are susceptible to penicillin by CLSI nonmeningitis breakpoints[1]; in contrast, only 67% of pneumococci are susceptible to penicillin by meningitis breakpoints. For penicillin, both meningitis and nonmeningitis interpretations should be reported, even for isolates from the respiratory tract or blood, as the laboratory infrequently receives information regarding whether the patient has signs and symptoms of meningitis.[2] Strains of *S. pneumoniae* that are susceptible to penicillin with an MIC of ≤ 0.06 µg/ml can be considered susceptible to other β-lactam agents. Even when pneumococci are penicillin nonsusceptible they can retain susceptibility to a later generation cephalosporin such as ceftriaxone, but this should be tested. Ceftaroline is active against the vast majority of *S. pneumoniae*, including penicillin and ceftriaxone resistant isolates, as it maintains high affinity for the mutant PBP 2X of these isolates.[3] Resistance rates for fluoroquinolones have remained low at < 5%,[1] but is more frequent in elderly patients.[4]

In contrast, macrolide susceptibility among pneumococci is low in the U.S. and in 2014, only 52.5% of isolates were susceptible to erythromycin based on a large US survey.[1] As such, testing for erythromycin resistance, which predicts activity of azithromycin, clarithromycin, and dirithromycin, should be done if macrolides are prescribed as monotherapy. CLSI recommends primary testing and reporting of penicillin, erythromycin, and TMP-SMX for isolates from non-CSF sources. Erythromycin and TMP-SMX can be tested by either an MIC or a disk diffusion method.[2] For penicillin, an MIC test can be done or an oxacillin 1 µg disk can be used to detect penicillin susceptibility. When an oxacillin zone of ≥ 20 mm is obtained, penicillin can be reported as susceptible. However, for strains with oxacillin zones ≤ 19 mm, a penicillin MIC must be subsequently performed prior to reporting penicillin results, as these isolates may be either resistant or susceptible. Testing of penicillin, cefotaxime or ceftriaxone by an MIC method, and testing of vancomycin, a fluoroquinolone, and tetracycline or doxycycline by an MIC method or disk diffusion would be appropriate for strains isolated from blood or respiratory sources. Although CLSI describes a test for inducible clindamycin resistance in *S. pneumoniae*, clindamycin is rarely indicated for treating respiratory pneumococcal infections other than otitis media.

More than 90% of *M. catarrhalis* isolates produce β-lactamase and are resistant to amoxicillin, ampicillin, and penicillin.[5] These isolates remain susceptible to amoxicillin-clavulanic acid, which is often prescribed for *M. catarrhalis* infections. Resistance to macrolides and tetracyclines is generally low (< 1% from isolates in the Western Hemisphere).[6] The cefinase disk method reliably detects β-lactamases produced by *M. catarrhalis*. Routine β-lactamase testing may not necessary because of the high incidence of β-lactamase-positive strains. Some advocate reporting of β-lactamase results to highlight the fact that this pathogen is generally unresponsive to some agents commonly prescribed for the treatment of respiratory tract infections (eg, amoxicillin).

Ampicillin resistance in *H. influenzae* can be due to expression of a plasmid-borne β-lactamase, or mutations to penicillin binding proteins (ie, β-lactamase negative, ampicillin resistant [BLNAR] strains). Compared with β-lactamase-producing or ampicillin-susceptible *H. influenzae*, BLNAR isolates have higher MICs to amoxicillin-clavulanic acid (which CLSI recommends reporting as resistant, regardless of MIC) and cephalosporins.[7] Some strains may possess both PBP mutations and a β-lactamase. These strains are referred to as β-lactamase-producing, amoxicillin-clavulanic acid resistant or BLPACR.[8] The current incidence of BLNAR and BLPACR isolates is relatively unknown—although studies in Canada demonstrated 16.4% of isolates recovered between 2007-2014 were resistant to ampicillin due to β-lactamase, another 14.6% were BLNAR, and 2.3% were BLPACR [1].Resistance among *H. influenzae* isolates to both broad-spectrum oral[7] and extended-spectrum cephalosporins (eg, ceftriaxone)[5,7] and fluoroquinolones remains rare among *H. influenzae*.[5] Because ampicillin is a treatment of choice if the isolate is susceptible, testing of *H. influenzae* by both a nitrocefin test (for β-lactamase) and by

AST of Bacteria Associated With Community-Acquired Pneumonia (*Continued*)

MIC or disk for ampicillin susceptibility (to rule out BLNAR strains) is recommended. If β-lactamase positive, amoxicillin-clavulanic acid is a treatment option; however, due to the possibility of BLPACR, this antimicrobial should be tested. For BLNAR isolates, a cephalosporin is often prescribed, or if the patient cannot tolerate β-lactam therapy, a fluoroquinolone or trimethoprim-sulfamethoxazole may be used.

One primary challenge in deciding when to perform susceptibility testing for *S. pneumoniae, H. influenzae,* and *M. catarrhalis* is that these organisms on a respiratory culture may represent colonization, and not infection. As such, testing should be restricted to institutional policies, generally when isolated in pure or near-pure culture, or as the predominant (eg, 3+ growth) potential pathogen.

Laboratories should work closely with their stewardship team to determine when testing is indicated and which antimicrobial agents should be tested.

Table 1. Suggestions for routine susceptibility testing of common bacterial pathogens causing CAP

Pathogen	Blood	Respiratory	Other agents to consider
S. pneumoniae	Penicillin (MIC) ESC Tetracycline/doxycycline Erythromycin	Penicillin (MIC or oxacillin disk) ESC Tetracycline/doxycycline Erythromycin	On request, vancomycin linezolid Fluoroquinolone TMP/SMX
M. catarrhalis	β-lactamase test (optional)	β-lactamase test (optional)	On request, macrolide tetracycline and other antimicrobials
H. influenzae	β-lactamase test Ampicillin MIC/Disk (if β-lactamase negative) ESC	β-lactamase test Ampicillin MIC/Disk (optional, if β -lactamase negative)	On request, ESC, fluoroquinolone and other antimicrobials

Abbreviation: ESC, extended-spectrum cephalosporin (eg, ceftriaxone, ceftaroline); MIC, minimum inhibitory concentration.

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Hot Topic

Candida auris

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A 76-year-old woman presents to the Emergency Department with fever and cough. The cough is productive of thick, bloody sputum and the lung examination reveals rales in lung bases. The patient has a history of congestive heart failure, diabetes, and recent hospitalization requiring broad-spectrum antimicrobial agents during the past 6 months. The patient is admitted to the ICU for respiratory failure and started on mechanical ventilation. Blood cultures and respiratory cultures are obtained. A central line and Foley catheter are inserted and the patient is started on cefepime plus vancomycin.

On day 2 after admission, the blood cultures are positive and demonstrate oval and elongated budding yeast forms on Gram stain. With identification pending, the patient is started on micafungin. The preliminary identification is *Candida guilliermondii.* The isolate is sent for confirmation of identification using molecular methods and reference susceptibility testing. Results from the reference laboratory reveal *Candida auris* with the following MIC profile:

MIC (µg/mL)	
Anidulafungin	1
Caspofungin	0.5
Fluconazole	>128
Micafungin	0.12
Voriconazole	1
Amphotericin B	1

Candida auris is an emerging public health threat due to elevated MIC values to multiple antifungal agents, potential for associated inter- and intra-hospital transmission, and persistence on fomites in the healthcare environment.[1,2] This *Candida* species was first reported in 2009 from patients in Japan and was followed by a report of an outbreak of this species in Korean hospitals.[3,4] Since its initial description, *C. auris* has been reported in multiple countries from all continents and has been associated with various outbreaks.[1]

C. auris can cause a variety of infections with severity that can range from mucosal infections to candidemia and it has similar virulence factors to *C. albicans*.[5] The risk factors for *C. auris* are similar to those of other *Candida* infections and include prolonged ICU stay, underlying respiratory illness, vascular surgery, prior antifungal exposure, and hospitalization.[6]

The identification of *C. auris* is challenging and conventional identification methods or biochemistry-based commercial identification systems are unable to correctly identify this species. Refer to **this** website for the recent recommendations from the CDC regarding identification of *C. auris*. In a recent report 10 *C. auris* and five isolates from other species that are commonly misidentified as *C. auris* were tested using four commercial phenotypic biochemical identification methods and two MALDI-TOF MS systems using different analysis databases and sample preparation methods.[7] None of the 10 *C. auris* isolates were correctly identified using the phenotypic systems and only the research use only (RUO) MALDI libraries were able to identify this species reliably. *C. auris* is closely related to *C. haemulonii* and often misidentified as the latter. Additionally, different methods might misidentify *C. auris* as *Candida famata, C. lusitaniae, C. parapsilosis, C. guilliermondii,* or *Rhodotorula glutinis*. Identification of the *Candida* species listed above (or *R. glutinis*) should prompt further investigation by laboratories to rule out *C. auris*. Correct identification of *C. auris* is possible by sequencing of the internal transcribed spacer and D1/D2 regions. Although correct identification by MALDI is not currently attainable with the FDA-cleared databases (due to absence of *C. auris* in these databases), *C. auris* may be identified correctly with use of research use only (RUO) libraries with the full tube extraction method. Variable results have been obtained with the use of direct on-plate extraction. Alternatively, sequencing of the isolate can be performed. The CDC has prepared a panel of *C. auris* isolates (and related species) available free of charge to aid laboratories in assessing their ability to recognize *C. auris* and for aid in validating their identification methods. The panel is available through the FDA-CDC Antimicrobial Resistance Isolate Bank which is described **here.**

Due to these challenges, the development of alternative methods for identification of *C. auris* has been proposed. Among those, one group proposed use of a CHROMagar medium supplemented with Pal's agar which is not commercially available but can be made in-house.[8] Nucleic acid-based assays on isolated colonies that have very high accuracy for *C. auris* identification have also been developed.[9]

Candida auris (*Continued*)

C. auris typically, but not invariably, display high fluconazole (> 64 µg/mL) and amphotericin (> 1 µg/mL) MICs and isolates exhibiting elevated echinocandin MIC values (> 0.5 µg/mL) have been reported. This means that these organisms are usually resistant to two or all three antifungal agent classes and can be difficult to treat. Not all isolates of *C. auris* display multidrug resistance. Synergy assays have been performed showing that echinocandins and amphotericin B combination could inhibit some of these isolates *in vitro*.[10]

Healthcare professionals need to be aware of the possibility of quick dissemination of *C. auris,* the importance of identification of *Candida* to the species level, and challenges in identifying *C. auris* organisms.

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