

# MicroFocus

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## A MACRO LOOK AT MICRO ISSUES

FALL 2006

### Introduction

Welcome to the second issue of MicroFocus, the information resource brought to you by MicroScan®, Dade Behring's line of microbiology products. The MicroFocus series is dedicated to spotlighting emerging issues in our complex, constantly changing field in order to help you keep abreast of the latest thinking and trends. Pursuant to that goal, we have enclosed a CD compilation of our 2006 ASM booth education sessions. We anticipate you will find these presentations both useful and timely, and will want to secure up to 5 CEU credits for completing the assessment test embedded in the CD.

This issue highlights antimicrobial agents from two perspectives. First, through discussion of the clinical utility of various classes of agents recommended for treatment of lower respiratory infections, and second through review and management of in vitro beta-lactam agent results for organisms possessing extended spectrum beta-lactamase enzymes.

Because we want to bring added value and establish an on-going dialogue with you, we have enclosed a reply card with this publication. You have the opportunity to obtain an additional hour of CEU credit if you complete the *Spotlight on ESBLs: A Continuing Laboratory Challenge* article evaluation and self-assessment test. The evaluation/self assessment may also be photocopied and returned to the same address on the reply card, enabling everyone in your lab the opportunity to earn credit. We have also provided space so you can let us know what other topics you would like explored in upcoming issues of MicroFocus. By completing and returning the card, you will help us continue to better meet your needs. Please email me directly if you would like to become an author for an upcoming publication. We hope you will find this second issue of MicroFocus as interesting and useful as the first.

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### Spotlight on ESBLs: A Continuing Laboratory Challenge

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Detection and reporting of bacteria that produce extended-spectrum beta-lactamases (ESBLs) present a continuing challenge for the clinical laboratory. This is due primarily to the complex nature of these enzymes and their increasing prevalence. The intent of this article is to provide a basic review of ESBLs and recommendations for detection and reporting of ESBL-producing gram-negative rods. In addition, we highlight the problems that may occur in the patient if ESBLs are not detected.

#### What are $\beta$ -lactams?

A group of antimicrobial agents that have a " $\beta$ -lactam ring" as part of their molecular structure. The antimicrobial classes included

*continued on page 2*

### Antimicrobial Overview From a Pharmacist Perspective: Lower Respiratory Infections

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Better practices and evidenced-based antimicrobial therapy recommendations have become the standard of care in many medical institutions providing care for patients. For example, community-acquired pneumonia (CAP) translates into having these recommendations available

in the emergency room and any other point of care entry into the hospital. The recommendations are often in the form of order sets and educational initiatives such as informative pocket cards,

CAP translates into having evidenced-based antimicrobial therapy recommendations available in the emergency room and any other point of care entry into the hospital.

*continued on page 7*

within the  $\beta$ -lactam group and a few examples of subclasses and specific agents are:

Class	Subclass	Examples
penicillins	aminopenicillins	ampicillin
	penicillinase-stable penicillins	oxacillin
$\beta$ -Lactam/ $\beta$ -Lactamase Inhibitors	none	amoxicillin-clavulanate piperacillin-tazobactam
cephems	cephalosporins 1st generation	cefazolin
	cephalosporins 3rd generation	cefotaxime
	cephamycins	cefoxitin
monobactams	none	aztreonam
penems	carbapenems	imipenem meropenem

### $\beta$ -lactams: Definitions

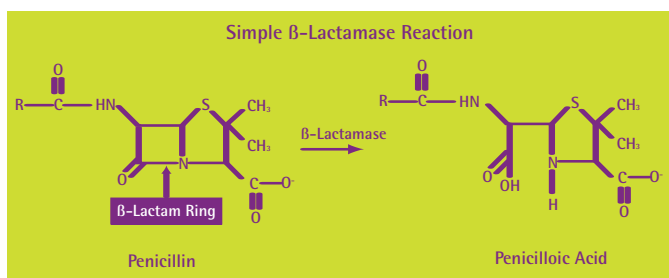
- Narrow-spectrum  $\beta$ -lactams
  - Active against gram-positive bacteria, eg, penicillin
- Broad-spectrum  $\beta$ -lactams
  - Active against gram-positive and gram-negative bacteria, eg, ampicillin, 1st-generation cephalosporins
- Extended-spectrum  $\beta$ -lactams
  - Active against gram-positive and enhanced activity against gram-negative bacteria
  - Extended-spectrum cephalosporins to include 3rd- and 4th-generation cephalosporins
  - Extended-spectrum penicillins to include carboxy- and ureidopenicillins, eg, ticarcillin and piperacillin, respectively

To obtain a full picture of the entire  $\beta$ -lactam group, check Glossary I in CLSI M100.<sup>1</sup>

### What is a $\beta$ -lactamase?

An enzyme that destroys the  $\beta$ -lactam ring portion of  $\beta$ -lactam molecules. Figure 1 shows a simple  $\beta$ -lactamase reaction depicting hydrolysis of penicillin to penicilloic acid. Penicilloic acid does not have antibacterial activity.

Figure 1



### What is an extended-spectrum $\beta$ -lactamase (ESBL)?

A type of  $\beta$ -lactamase that hydrolyzes (inactivates) most extended-spectrum  $\beta$ -lactams (but NOT cephamycins, carbapenems, or  $\beta$ -lactamase inhibitor combinations).

### 8 important facts about ESBLs

- Primarily found in *E. coli*, *Klebsiella* spp., and *P. mirabilis* but can be found in other gram-negative rods
- Arise from small mutations in genes (usually located on plasmids) that code for production of common  $\beta$ -lactamases (eg, TEM-1 that codes for ampicillin resistance in *E. coli*)
- Are inhibited by  $\beta$ -lactamase inhibitors (eg, clavulanic acid)
- Can cause serious infections
- Can cause nosocomial infections - failure to detect ESBLs has contributed to their uncontrolled spread
- Are often resistant to agents in other antimicrobial classes (eg, aminoglycosides, fluoroquinolones)
- Are generally susceptible to carbapenems and agents within this class (eg, imipenem or meropenem) which are often used to treat serious infections caused by ESBL producers
- Many types of ESBLs (approximately 300) have been described, and these contribute to a variety of susceptibility profiles

Let's look at varying cephalosporin MIC results ( $\mu\text{g/mL}$ ) for three isolates producing three different ESBLs.

Isolate	ESBL Type	Cephalothin 1st-gen cephalosporin	Cefotaxime 3rd-gen cephalosporin	Ceftazidime 3rd-gen cephalosporin
1	TEM-4	256	8	16
2	SHV-5	256	4	32
3	CTX-M-15	64	64	4

If ESBL-producing *Klebsiella pneumoniae* is isolated from a blood culture and the susceptibility test shows that it is "S" to cefotaxime, what could happen if we ignore ESBL reporting rules and report cefotaxime as "S"?

If cefotaxime is used to treat the patient, the patient may fail therapy and die. This is an example where *in vitro* results may NOT correlate with clinical outcome.

Here are data that examined the outcomes for 28 patients with serious ESBL infections when treated with a 3rd-generation cephalosporin (eg, cefotaxime). Over half the patients were not cured with the 3rd-generation cephalosporin, and four died.<sup>2</sup>

MIC (µg/mL) for a 3rd-generation cephalosporin such as cefotaxime and CLSI interpretation	Clinical Failure	Died Within 14 Days of Bacteremia
8 S	100% (6/6)	33% (2/6)
4 S	67% (2/3)	0 (0/3)
2 S	33% (1/3)	0 (0/3)
≤1 S	27% (3/11)	18% (2/11)
Recorded as ≤4 S	60% (3/5)	Not given
Total	54% (15/28)	n/a

### Why do ESBL producers sometimes test "S" to drugs and then these drugs do not work in the patient?

In order for an antimicrobial agent to cure an infection, the drug must 1) get to the site of the infection and 2) attain a concentration at the infection site that is greater than the MIC of the infecting bacterium. With standardized MIC tests, 10<sup>5</sup> CFU/mL are tested. Sometimes the concentration of bacteria at the infection site is greater than this. The MIC generally increases for an ESBL-producing isolate when challenged with greater than 10<sup>5</sup> CFU/mL. At the higher concentration of bacteria, the drug cannot overcome the increased concentration of ESBL that is present. Although this is one possible explanation, other factors may contribute to clinical failures, such as concentration of drug at the infection site compared with the MIC of the infecting bacteria and how long that concentration stays above the MIC.

### What does CLSI say about ESBL testing?

Labs should test *E. coli*, *Klebsiella* spp., and *Proteus mirabilis*. ESBL testing involves two steps:

**Step 1** is the ESBL screen test and **Step 2** is the ESBL phenotypic confirmatory test. A diagram depicting this process is shown in Figure 2, and the procedure for each step follows.

#### Step 1: ESBL screen test

##### Principle

An isolate that shows decreased susceptibility (small zone or elevated MIC) to one or more of the ESBL screening agents is suspicious for ESBL production.

#### Procedure

Test two or more of the following by disk diffusion or MIC: cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, aztreonam.

#### Results:

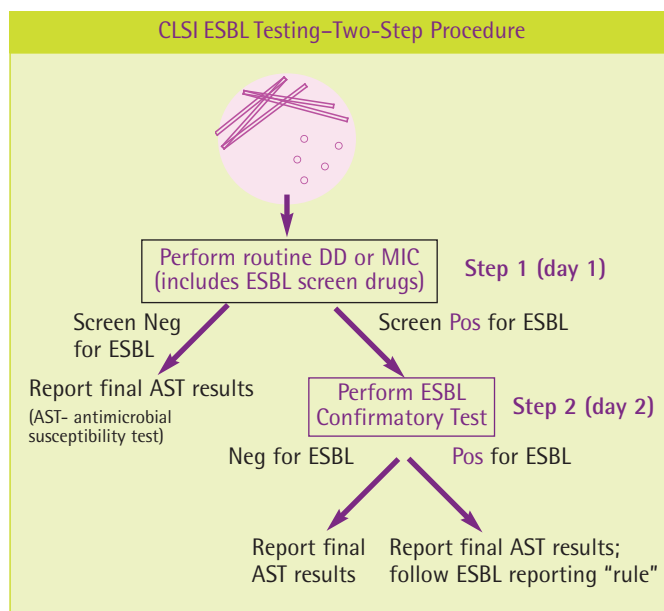
If isolate fits criteria listed here it is "suspicious" for ESBL production.

	Disk Zone (mm)	MIC (µg/mL)
*Cefpodoxime**	≤ 17	> 4
*Ceftazidime	≤ 22	> 1
*Cefotaxime	≤ 27	> 1
Ceftriaxone	≤ 25	> 1
Aztreonam	≤ 27	> 1

\*Only these drugs are appropriate for *P. mirabilis*.  
 \*\* ≤ 22 mm and >1 µg/mL is used for *P. mirabilis*.

If an isolate is suspicious for ESBL production based on the screen test, report preliminary results and then go to Step 2. If an isolate is suspicious for ESBL production, suppress any "S" result for penicillins, cephalosporins, and aztreonam until results for the ESBL confirmatory test are available. If the isolate is confirmed as an ESBL producer, these "S" results will be edited to "R".

Figure 2



#### Step 2: ESBL phenotypic confirmatory test

##### Principle

Clavulanic acid restores the activity of either cefotaxime, ceftazidime, or both for ESBL producers. Clavulanic acid, a β-lactamase inhibitor, prevents the ESBL β-lactamase

from hydrolyzing the drugs but has virtually no antibacterial activity.

### Procedure

Test the following agents by disk diffusion or MIC: cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid.

### Results

If clavulanic acid restores the activity of either cefotaxime, ceftazidime, or both, the isolate is considered to be an ESBL producer. Restoration of activity is defined as follows:

- Disk diffusion test—a 5 mm or greater increase in the zone diameter for cefotaxime (or ceftazidime) with clavulanic acid as compared to the zone diameter for cefotaxime (or ceftazidime) alone (Figure 3)
- MIC test—a  $\geq 3$  two-fold dilution drop in MIC for cefotaxime (or ceftazidime) with clavulanic acid as compared to the MIC for cefotaxime (or ceftazidime) alone (Figure 4)
- Important! Only ONE set of drugs needs to be positive to consider isolate an ESBL producer
- If ESBL production is confirmed, edit "S" result to "R" for any penicillin, cephalosporin, or aztreonam (see Table 1)
- If clavulanic acid does NOT restore the activity of cefotaxime or ceftazidime according to the criteria stated above, the isolate is considered ESBL negative and no results are edited

Figure 3

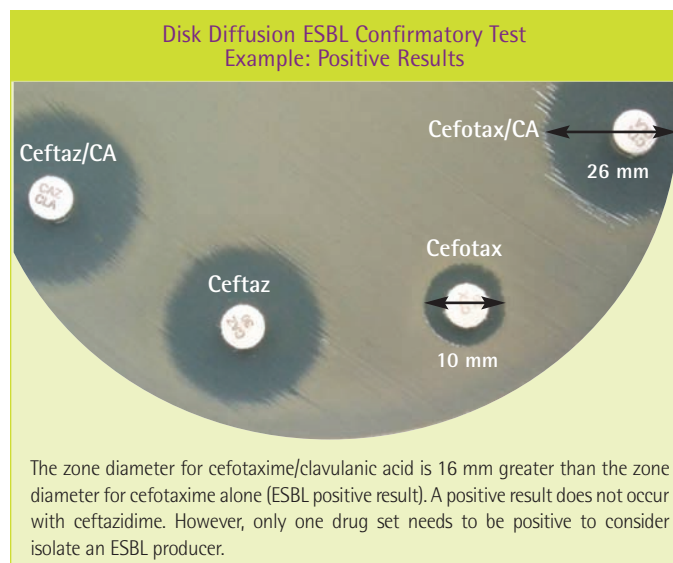


Figure 4

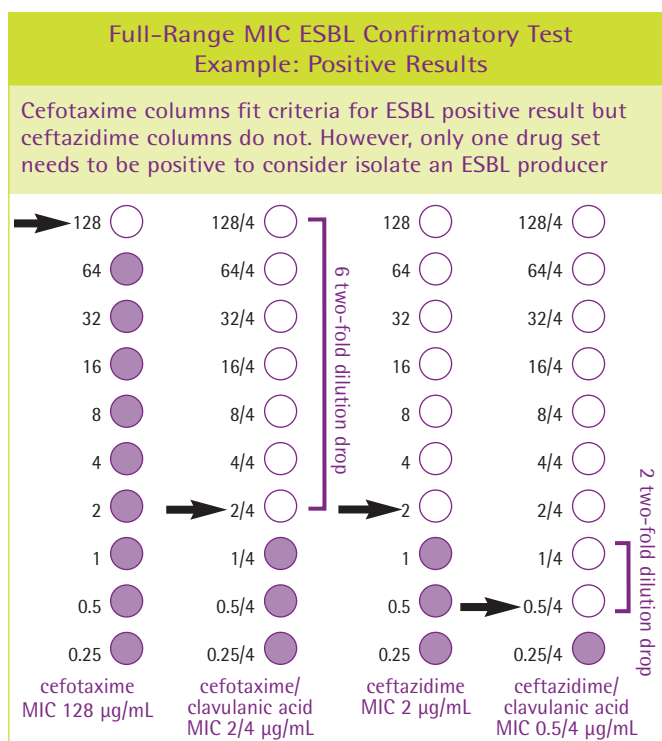


Table 1.

### Actions necessary if ESBL production is confirmed

If "S", edit to "R"		Do not edit to "R"	
Penicillins <sup>a</sup>	Cephalosporins <sup>a</sup>	Cephameycins	$\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations
ampicillin carbenicillin mezlocillin piperacillin ticarcillin	cephalothin cefazolin cefamandole cefuroxime cefotaxime ceftazidime ceftizoxime ceftriaxone cefepime ...aztreonam (monobactam)	cefoxitin cefotetan cefmetazole	amoxicillin-clavulanic acid ampicillin-sulbactam piperacillin-tazobactam ticarcillin-clavulanic acid

<sup>a</sup>These are the most common penicillins and cephalosporins that might be tested on a gram-negative panel. However, ALL penicillins and cephalosporins listed in CLSI M100 Glossary should be considered "R" for ESBL procedures.<sup>1</sup>

Final report example: <i>Klebsiella pneumoniae</i>	Notes
ampicillin >32 R	Cefepime and cefotaxime that had MICs in the "S" range of 1 and 4 µg/mL, respectively, are now reported as "R".
cefazolin >32 R	
cefepime R	A comment may be added to the report, if desired, such as "Confirmatory tests for this <i>K. pneumoniae</i> indicate unusual resistance [extended-spectrum beta-lactamase (ESBL) production]"
cefotaxime 1 S	
ciprofloxacin R	
gentamicin 0.5 S	
imipenem >16 R	
trimethoprim-sulfamethoxazole $\leq 0.25$ S	
piperacillin-tazobactam $\leq 8/4$ S	
trimethoprim-sulfamethoxazole >4/76 R	
trimethoprim-sulfamethoxazole	

## Quality-control organisms

For daily or weekly QC, test the following:

- ESBL screen test—*E. coli* ATCC 25922 OR *K. pneumoniae* ATCC 700603
- ESBL phenotypic confirmatory test—*E. coli* ATCC 25922 AND *K. pneumoniae* ATCC 700603

*K. pneumoniae* ATCC 700603 is an ESBL-producing strain. The ESBL gene is located on a plasmid (extra-chromosomal DNA). Plasmids can be easily lost from bacteria if the bacteria are not properly maintained. To avoid problems with *K. pneumoniae* ATCC 700603:

- 1) Store permanent stock cultures at temperatures at or below -60°C
- 2) Store working stock culture on an agar slant for up to one month
- 3) Subculture from the agar slant weekly to obtain colonies to use for inoculum preparation

For acceptable QC results, check Table 3 in CLSI M100.<sup>1</sup>

## What is MicroScan's "streamlined" ESBL confirmatory test?

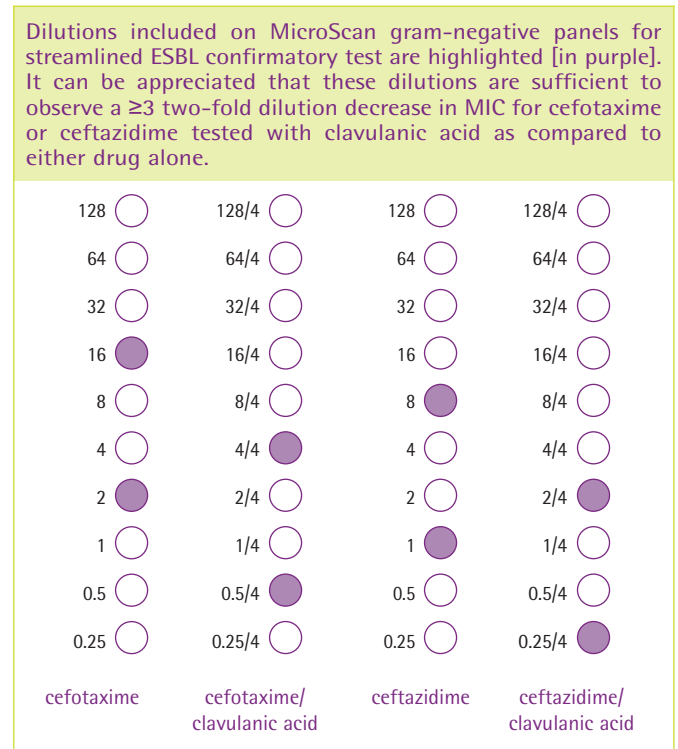
- An abbreviated version of the CLSI MIC confirmatory test that is included on some of MicroScan routine gram-negative panels. The concentrations included are shown in Figure 5
- "Low" and "high" dilutions of cefotaxime and ceftazidime with and without clavulanic acid are tested. If there is growth in the high-dilution well, the MIC is greater than the concentration of drug in that well. If there is no growth in the low-dilution well, the MIC is less than the concentration of drug in that well
- The abbreviated dilution scheme enables assessment of a  $\geq 3$  two-fold dilution decrease in MIC for cefotaxime or ceftazidime tested in combination with clavulanic acid as compared to the MIC for either drug alone. An example is shown in Figure 6

## What are the advantages of MicroScan's "streamlined" ESBL confirmatory test?

- A separate ESBL confirmation test is not needed
- ESBL test results are available at the same time that routine MIC results for the panel are available
- Detection of ESBLs is a one-step, not a two-step, procedure

- All of the above contribute to less work and more rapid results

Figure 5



## What are some pitfalls of the current CLSI ESBL test?

- Some isolates may have ESBLs plus other resistance mechanisms that may mask ESBL detection in the confirmatory test
- ESBLs occur in species other than *E. coli*, *Klebsiella* spp., and *Proteus mirabilis*, which CLSI does not currently address

Figure 6

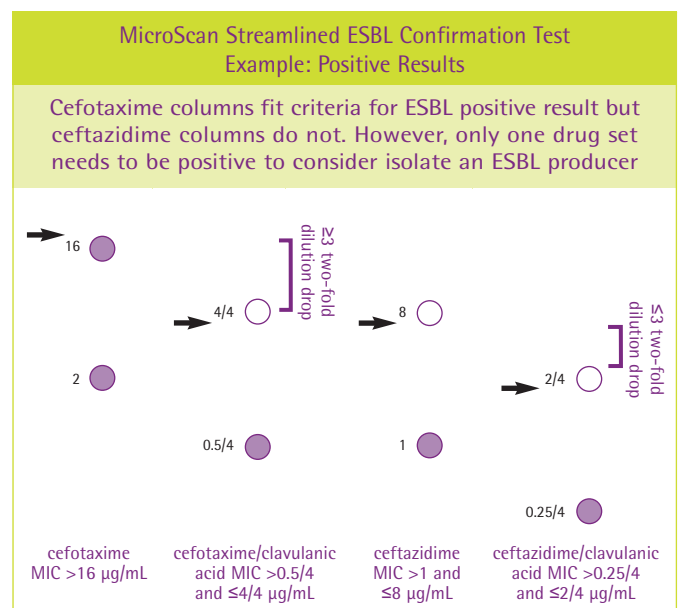


Table 2 shows growth patterns possible in test wells and interpretation of results for MicroScan's streamlined ESBL confirmatory test. **Remember, only one set of drugs (cefotaxime or ceftazidime set) needs to be positive to confirm ESBL production. Most ESBL producers will show both sets positive!**

**Table 2 Cefotaxime Set**

Low Dilutions		High Dilutions		ESBL Interpretations	Rationale for Interpretation
Cefotaxime 2 µg/mL	Cefotaxime/CA 0.5/4 µg/mL	Cefotaxime 16 µg/mL	Cefotaxime/CA 4/4 µg/mL		
No growth	No growth	No growth	No growth	Negative	"S" to cefotaxime
Growth	Growth	Growth	Growth	Negative	1. CA does not adequately restore activity of cefotaxime. 2. Possible ESBL, unable to interpret confirmation test. Organism has MICs > highest dilution on panel. Streamlined ESBL confirmation test will be reported as negative.
Growth	Growth	No growth	No growth	Negative	
Growth	No growth	Growth	No growth	Positive	CA restores activity of cefotaxime (≥3 two-fold dilution decrease in MIC with CA).
Growth	No growth	No growth	No growth	Positive	
Growth	Growth	Growth	No growth	Positive	

### Self-assessment questions

To check your understanding of ESBL testing and reporting, please answer the following 5 self-assessment questions.

**1. Extended-spectrum β-lactamases are most commonly found in:**

- a. *E. coli* and *Klebsiella* spp.
- b. *E. coli*, *Klebsiella* spp., and *P. mirabilis*
- c. *E. coli* and *Enterobacter cloacae*
- d. *E. coli* and *Pseudomonas aeruginosa*
- e. *Klebsiella* spp. and *P. mirabilis*

**2. According to CLSI rules, which of the following are NOT edited to "R" for ESBL producers?**

- a. Aztreonam
- b. Cefepime
- c. Cefotaxime
- d. Ceftriaxone
- e. Piperacillin-tazobactam

**3. What is the function of clavulanic acid in the ESBL confirmatory test?**

- a. It stimulates production of the ESBL
- b. It decreases production of the ESBL
- c. It blocks the ESBL and prevents it from hydrolyzing cefotaxime or ceftazidime
- d. It has very potent antibacterial activity against the test organism
- e. It decreases the size of the bacterial cells

**4. Which of the following is NOT a characteristic associated with ESBL producing bacteria?**

- a. They cause serious, sometimes fatal infections
- b. They can cause nosocomial outbreaks
- c. They are often multiply resistant to several classes of antimicrobial agents
- d. Genes that code for ESBL production are generally located on plasmids
- e. They are easily detected with routine disk diffusion or MIC tests for cefotaxime and ceftazidime

**5. Which of the following would be considered ESBL positive?**

	MIC (µg/mL)			
	Cefotaxime	Cefotaxime/CA	Ceftazidime	Ceftazidime/CA
a	>32	>32/4	32	16/4
b	≤2	≤0.5/4	≤1	≤0.25/4
c	32	≤0.5/4	16	≤0.25/4
d	>32	32/4	>32	16/4
e	≤2	≤0.5/4	4	1/4

**Answers will be published in the next issue of MicroFocus.**

**Table 2 (cont.) Ceftazidime Set**

Low Dilutions		High Dilutions		ESBL-Interpretations	Rationale for Interpretation
Ceftazidime 2 µg/mL	Ceftazidime/CA 0.5/4 µg/mL	Ceftazidime 16 µg/mL	Ceftazidime/CA 4/4 µg/mL		
No growth	No growth	No growth	No growth	Negative	"S" to ceftazidime
Growth	Growth	Growth	Growth	Negative	1. CA does not adequately restore activity of ceftazidime. 2. Possible ESBL, unable to interpret confirmation test. Organism has MICs > highest dilution on panel. Streamlined ESBL confirmation test will be reported as negative.
Growth	Growth	No growth	No growth	Negative	
Growth	No growth	Growth	No growth	Positive	CA restores activity of ceftazidime (≥3 two-fold dilution decrease in MIC with CA).
Growth	No growth	No growth	No growth	Positive	
Growth	Growth	Growth	No growth	Positive	

**References:** 1. Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. M100-S16. CLSI, Wayne, PA. 2. Paterson, D.L., et al. 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. *J Clin Microb*. 39:2206-2212.

## Antimicrobial Overview From a Pharmacist Perspective: Lower Respiratory Infections

*continued from page 1*

posters, and lectures with compliance monitoring to provide feedback. For healthcare-associated pneumonia (HAP), clinical pathways incorporating diagnostic tools and antibiotic recommendations are often based on the individual institution's current practices for respiratory specimen collection for culture (qualitative versus quantitative) and the institution's intensive care unit (ICU) microbial epidemiology and cumulative antibiogram.

### COMMUNITY-ACQUIRED PNEUMONIA (CAP)

Typical antibiotics used in the hospital for CAP include second- and third-generation fluoroquinolones, advanced macrolides, tetracyclines, and third-generation cephalosporins. In reviewing each of the classes for this indication, it becomes important to remember the most common pathogens in CAP are *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, and the atypicals *Legionella pneumophila*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

**Fluoroquinolones:** Quinolones used to treat respiratory infections exhibit activity against all the pathogens suspected in CAP. Second-generation quinolones such as levofloxacin have significant activity against gram-negative bacilli, gram-positive cocci including *S. pneumoniae*, and atypicals. Moxifloxacin is a third-

generation quinolone with improved activity against gram-positive organisms and expands to include anaerobes. The mechanism of action for the quinolones is inhibition of nucleic acid synthesis by binding to DNA gyrase.<sup>1</sup> All things considered, formulary considerations for the quinolones primarily center on cost and their restricted use in pediatrics.

**Advanced macrolides:** Clarithromycin and azithromycin are preferred macrolides for patients hospitalized with CAP versus erythromycin due to better pharmacokinetic, enhanced spectrum of activity, and tolerability profiles. Azithromycin is available in intravenous and oral formulation administered once daily and is used in combination regimens for CAP primarily for its excellent activity against atypical pathogens.<sup>2</sup> Additionally, *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* are all mostly susceptible to azithromycin. In areas of high macrolide-resistant *S. pneumoniae*, combination antibiotics are warranted. The mechanism of action for the macrolides is reversible binding to the 50S ribosomal subunit inhibiting protein synthesis.<sup>3</sup>

**Tetracyclines:** Tetracyclines are broad-spectrum antibiotics with activity against gram-positive and gram-negative bacteria, rickettsiae, and atypical CAP pathogens such as *Chlamydia* and *Mycoplasma*. The most common tetracycline derivatives used in hospitals include doxycycline and minocycline which are both available in oral and intravenous formulations. Additionally, these two agents exhibit less adverse side effects than tetracycline and

require fewer dose administrations per day. The mechanism of action of tetracycline is the inhibition of protein synthesis following binding to the 30S ribosomal subunit. When coupled with a third-generation cephalosporin with activity against *S. pneumoniae*, this combination offers appropriate coverage for inpatient CAP therapy.<sup>4</sup>

**Cephalosporins:** Third- and fourth-generation cephalosporins are commonly used for community acquired and healthcare associated pneumonias. Cephalosporins with excellent *S. pneumoniae* activity include cefotaxime and ceftriaxone. Cephalosporins with good activity against *Enterobacteriaceae* and *Pseudomonas aeruginosa* include ceftazidime, with cefepime exhibiting equivalent potency and additionally covering *S. pneumoniae*.<sup>5</sup>

### HEALTHCARE-ASSOCIATED PNEUMONIA (HAP)

HAP is complicated by the recovery of multidrug-resistant (MDR) pathogens typically including methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and *Acinetobacter* spp. [see Figure 1 for a typical ICU ventilator-associated pneumonia (VAP) pathway]. One of the most important approaches to creating a HAP pathway is to use a collaborative effort with a multidisciplinary team including key players from areas such as respiratory therapy, infection control, infectious diseases, nursing, microbiology, and pharmacy.

The critical selection of empiric antimicrobials must be made based on local epidemiology and antibiograms in addition to incorporating pharmacokinetic/pharmacodynamic principles with antimicrobial toxicities. ICU antibiograms of respiratory isolates are often useful in the selection of the most appropriate empiric agents. In vitro susceptibility trending of four antipseudomonal antimicrobials from isolates recovered in the ICU versus the overall hospital population clearly demonstrates a high level of *P. aeruginosa* resistance to fluoroquinolones (see Figure 2). Therefore, this class of antimicrobials would not be recommended for empiric antimicrobial guidelines for VAP pathways in our ICU. Common classes of antibiotics utilized for HAP include extended-spectrum cephalosporins,  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations, aminoglycosides, glycopeptides, antipseudomonal fluoroquinolones, and linezolid. If pan-resistant *Acinetobacter* species is a problem in your institution, tigecycline and colistin should also be tested.

**$\beta$ -lactam/ $\beta$ -lactamase inhibitors:** There are three parenteral inhibitor combinations commonly used in clinical practice: ampicillin-sulbactam, ticarcillin-clavulanate, and piperacillin-tazobactam. Due to the broad-spectrum activity of this class of antibiotics against aerobic and anaerobic  $\beta$ -lactamase-producing organisms, these agents are commonly utilized for empiric coverage when polymicrobial infections are suspected.<sup>6</sup> The potency of each  $\beta$ -lactam agent varies, with piperacillin-tazobactam exhibiting the best activity against *Pseudomonas aeruginosa*. The mechanism of action, as with other penicillins, is inhibition of cell wall synthesis following binding to penicillin-binding proteins. Clinical efficacy is achieved for a beta-lactam antibiotic when the concentration of the antibiotic at the site of the infection exceeds the MIC for approximately 50% of the dosing interval. In serious health care associated pneumonias, higher doses (4.5 gram IV every 6 hours) are recommended to achieve this goal.<sup>7,8</sup>

**Carbapenems:** Imipenem and meropenem are among the broadest antimicrobial agents with activity against aerobic gram-positives including methicillin-sensitive *Staphylococcus aureus*, pneumococci, and streptococci, in addition to gram-negative bacteria as well as anaerobes. Key coverage characteristics for imipenem are most probably due to the following: its ability to penetrate the cell wall of gram-negative rods, its affinity for a wide variety of penicillin-binding proteins, and its resistance to most  $\beta$ -lactamases found in both gram-positive and gram-negative bacteria. As a rule, imipenem is slightly more potent against gram-positives and meropenem is slightly more potent against gram-negatives.<sup>9</sup> In serious *Pseudomonas* species infections, most experts would use combination therapy of a carbapenem with a synergistic aminoglycoside or an antipseudomonal quinolone (ciprofloxacin or high-dose 750 mg levofloxacin). However, there is a recent report of carbapenem discordant *Acinetobacter* species susceptible to imipenem and resistant to meropenem with the mechanism attributable to efflux pumps.<sup>10</sup>

**Aminoglycosides:** The spectrum of activity for the aminoglycosides primarily includes gram-negative bacilli, with gentamicin also exhibiting synergistic activity against gram-positive bacteria and amikacin having additional coverage for mycobacteria. The mechanism of action is inhibition of protein synthesis following binding to the 30S ribosome. Dosing strategies are either conventional intermittent infusion or once-daily aminoglycoside

dosing (ODD). ODD is used in an attempt to maximize the concentration-dependent pharmacodynamic principle of 10 times the MIC for rapid killing. Gentamicin or tobramycin are routinely combined with  $\beta$ -lactams for serious gram-negative treatment in the adult and pediatric population.

**Glycopeptide:** Vancomycin exhibits activity against gram-positive organisms through inhibition of cell wall synthesis. Vancomycin is used routinely for methicillin-resistant *Staphylococcus aureus* infections and may commonly be found as an empiric recommendation for skin and soft tissue infections and in combination with other antibiotics for nosocomial *S. aureus* pneumonias. A typical empiric dosing strategy is intravenous vancomycin 15 mg/kg of patient body weight given at a frequency determined by renal elimination. Therapeutic drug monitoring is utilized to confirm target trough levels of 5-15  $\mu\text{g/mL}$ . Tolerance to vancomycin has been recently demonstrated in *Staphylococcus* species with pharmacodynamic principles of vancomycin in addition to higher staph MICs considered attributable.<sup>11</sup>

**Oxazolidinone:** Linezolid's spectrum of activity includes gram-positive organisms such as vancomycin-resistant enterococci (VRE), penicillin-resistant *S. pneumoniae*, and methicillin-resistant *Staphylococcus aureus*. FDA-approved indications include VRE bacteremia, complicated and uncomplicated skin and skin structure infections, community-acquired and nosocomial pneumonias.<sup>12</sup> However, due to high acquisition costs and considerably more experience with vancomycin, linezolid is often reserved for patients with intolerance to vancomycin or when intravenous administration is problematic. Furthermore, linezolid should be reserved

for cases where MDR gram-positive pathogens are suspected.<sup>13</sup> The mechanism of action is inhibition of protein synthesis. Linezolid is available in oral capsules, oral solution, and intravenous formulation with the bioavailability of oral formulations approaching 100%.

**Polymyxins:** Polymyxins were first introduced around 1950 with a reformulation of colistin as Coly-Mycin around 1960. Mechanism of action is direct binding to the cell membrane resulting in permeability changes and ultimate cell death. The spectrum of activity is exclusively gram-negative bacilli including MDR pathogens such as *Pseudomonas* and *Acinetobacter* spp. Colistin is often the last line of therapy for pan-resistant strains and may be administered both by parenteral route and adjunctively by aerosolization in ventilator associated pneumonia cases.

**Glycylcycline:** Tigecycline is a minocycline derivative and the first in this class of antibiotics. The spectrum of activity is very broad and includes methicillin-resistant *S. aureus*, penicillin-resistant *S. pneumoniae*, anaerobes, atypicals, *Enterobacteriaceae* including ESBLs, and non-lactose fermenting gram-negative bacilli such as *Acinetobacter* spp. (some pan-resistant strains) and *S. maltophilia*. Efflux pumps present in *Pseudomonas aeruginosa* and *Proteus mirabilis* confer intrinsic resistance to tigecycline. Tigecycline has been studied in complicated skin and soft tissue and intra-abdominal infection trials. Community and hospital acquired pneumonia trials are currently in progress. The clinical utility of tigecycline may be most beneficial for mixed surgical wound infections and a viable option for multidrug-resistant *Acinetobacter* spp. isolates resistant to all classes of antibiotics except the polymyxins and aminoglycosides.<sup>14,15</sup>

**Figure 1: Ventilator-associated pneumonia (VAP) pathway:**

Obtain mini-BAL from intubated patients for quantitative culture & stat gram stain

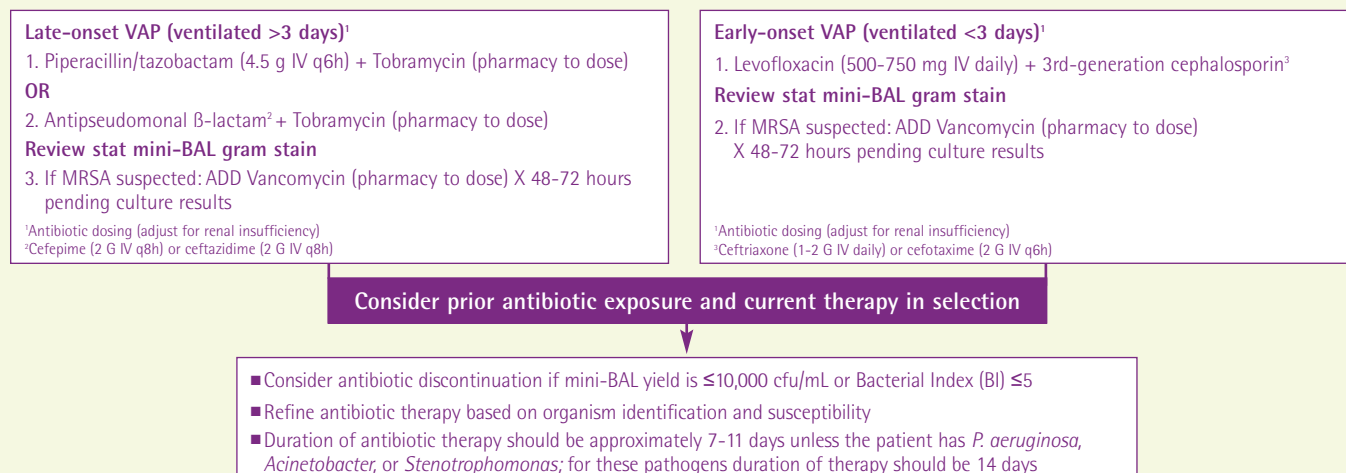
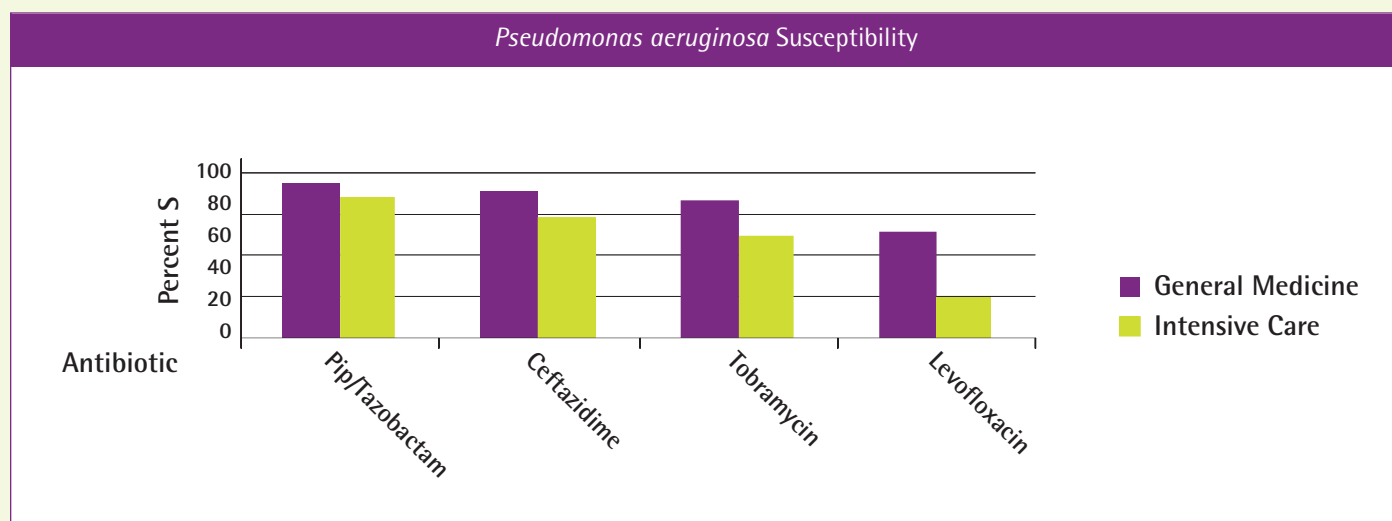


Figure 2: *P. aeruginosa* trending.



Reference: 2004 Antibiogram data, Columbus Regional Healthcare System

Table 1: Antimicrobial agent classification and mode of action.

Class	Examples	Mode of Action
aminoglycoside	gentamicin, tobramycin, amikacin	Bactericidal; Bind to 30S ribosomal subunit and inhibit protein synthesis
β-lactam/β-lactamase inhibitors	ampicillin-sulbactam, ticarcillin-clavulanate, piperacillin-tazobactam	Bactericidal; Inactivate penicillin-binding proteins to inhibit cell wall synthesis
carbapenem	imipenem, meropenem, ertapenem	Bactericidal; Cell wall elongation and lysis
cephalosporin (3rd and 4th generation)	cefotaxime, ceftriaxone, ceftazidime, cefepime	Bactericidal; Inactivate penicillin-binding proteins to inhibit cell wall synthesis
fluoroquinolone	ciprofloxacin, levofloxacin, moxifloxacin	Bactericidal; Bind to bacterial DNA gyrase and block DNA replication
glycopeptide	vancomycin	Bactericidal; Inhibition of cell wall synthesis
glycylcycline	tigecycline	Bacteriostatic; Bind to bacterial ribosome to inhibit protein synthesis
macrolide	erythromycin, clarithromycin, azithromycin	Bacteriostatic; Bind to 50S ribosome and inhibit protein synthesis
oxazolidinone	linezolid	Bacteriostatic; Bind to 50S ribosome and inhibit protein synthesis
polymyxins	polymyxin B, colistin	Bactericidal; Disrupt cell membrane
tetracycline	tetracycline, doxycycline, minocycline	Bacteriostatic; Binds to 30S ribosomal subunit and inhibit protein synthesis

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## Commentary



William J. Brown, Ph.D., D(ABMM), F(AAM)

MicroScan®, a product line of Dade Behring, maintains their commitment to continuing education of laboratorians with this second installment of the MicroFocus newsletter. The articles in this issue address antimicrobial agents

from two aspects: a pharmacist's view on antibiotics as used in the therapy of pneumonia and an overview for laboratorians on ESBL basics with the latest update on ESBL detection available from MicroScan.

Deanne Tabb, as a pharmacist, discusses use of data generated in the microbiology lab to guide patient therapy. She also describes the use of cumulative antibiogram data when developing empiric therapy recommendations that are included in "Clinical Pathways" for treating infectious diseases. Sometimes unit-specific (eg, ICU) cumulative antibiogram data are helpful in fine tuning empiric therapy recommendations. Clinical pathways provide guidance for physicians when managing patients with various diseases. One or more representatives of the clinical microbiology department should be actively involved in development of clinical pathways for infectious diseases. Dr. Tabb uses pneumonia as an example in her discussion of classes of antimicrobial agents and, more specifically, the "pathway" for treatment of patients with ventilator-associated-pneumonia (VAP). Technologists are expected to be knowledgeable of the antimicrobial classes and limitations of *in vitro* testing for certain drugs or classes of drugs when testing various organisms. Ensuring accurate data for individual patient reports as well as for the cumulative antibiogram reports must be a primary objective of every laboratory worker.

The accurate and sensitive detection of resistance is vital for good clinical outcomes, particularly in patients with serious infections. The sooner the patient can be placed on therapy directed towards the specific bacterium causing the infection, the greater likelihood of a positive clinical outcome. For example, one study has shown that if a patient is treated empirically with ceftazidime for septicemia resulting from an ESBL-positive *Klebsiella pneumoniae*, a delay of greater than 72 hours in

detection of the ESBL enzyme activity will result in three-fold increase in mortality.<sup>1</sup> The timely and accurate detection of ESBLs in *Enterobacteriaceae* recovered from bloodstream infections is essential, with another study demonstrating that death from infection doubles for infections caused by strains that are ESBL positive as compared with those that are ESBL negative (30% vs 16%).<sup>2</sup> The number of different types of ESBLs is rapidly increasing and spreading among gram-negative bacilli. By the end of 2005, 291 different common types (TEM, SHV, and CTX-M) of ESBLs with varying activities had been described.<sup>3</sup> Studies also implicate transient carriage of ESBL-producing organisms on the hands of healthcare workers as an important means of patient-to-patient transfer, further supporting the need for timely detection and reporting to facilitate prudent patient-and infection-control management.<sup>4</sup>

Janet Hindler has provided basic facts about ESBLs and current recommendations for *in vitro* detection of these complex enzymes. She summarizes the CLSI reference procedures and introduces us to the new MicroScan "streamlined" ESBL confirmation test. This test is FDA-cleared for detecting ESBL-producing *E. coli*, *Klebsiella* species, and *P. mirabilis*. The streamlined ESBL confirmation test is on several MicroScan routine gram-negative panels so ESBL testing is performed concurrently with MIC testing. Consequently, besides reducing the amount of work and supplies, results are available a day sooner than with conventional two-step ESBL tests. The new streamlined ESBL confirmation test will help to ensure the best clinical outcomes in our patients.

**References:** 1. Anderson, D.J. et al. 2006. Prediction of mortality in patients with bloodstream infection due to ceftazidime resistant *K. pneumoniae*. *Antimicrob Agents Chemother* 50: 1715 – 1720. 2. Schwaber, M.J., et al. 2006. Clinical and economic impact of bacteria with extended-spectrum-β-lactamase-producing *Enterobacteriaceae*. *Antimicrob Agents Chemother* 50: 1257 – 1262. 3. Jacoby, G.A. 2006. β-lactamase nomenclature. *Antimicrob Agents Chemother* 50: 1123 – 1129. 4. Paterson, D.L., et al. 2005. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 18: 657 – 686.

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