Controversies about Extended-Spectrum and AmpC Beta-Lactamases

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Many clinical laboratories have problems detecting extended-spectrum beta-lactamases (ESBLs) and plasmid-mediated AmpC beta-lactamases. Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate reporting conventions. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures. Although National Committee for Clinical Laboratory Standards recommendations exist for detecting ESBL-producing isolates of *Escherichia coli* and *Klebsiella* spp., no recommendations exist for detecting ESBLs in other organisms or for detecting plasmid-mediated AmpC beta-lactamases in any organisms. Clinical laboratories need to have adequate funding, equipment, and expertise to provide a rapid and clinically relevant antibiotic testing service in centers where these resistance mechanisms are encountered.

Extended-spectrum beta-lactamases (ESBLs) were first reported in 1983 (1), and plasmid-mediated AmpC beta-lactamases were reported in 1988 (2). Typically, ESBLs are mutant, plasmid-mediated beta-lactamases derived from older, broad-spectrum beta-lactamases (e.g., TEM-1, TEM-2, SHV-1), which have an extended substrate profile that permits hydrolysis of all cephalosporins, penicillins, and aztreonam. These enzymes are most commonly produced by *Klebsiella* spp. and *Escherichia coli* but may also occur in other gram-negative bacteria, including *Enterobacter, Salmonella, Proteus,* and *Citrobacter* spp., *Morganella morganii*, *Serratia marcescens*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Capnocytophaga ochracea* (3-9). Plasmid-mediated AmpC beta-lactamases have arisen through the transfer of chromosomal genes for the inducible AmpC beta-lactamase onto plasmids. This transfer has resulted in plasmid-mediated AmpC beta-lactamases in isolates of *E. coli, Klebsiella pneumoniae, Salmonella* spp., *Citrobacter freundii, Enterobacter aerogenes,* and *Proteus mirabilis* (10-12). To date, all plasmid-mediated AmpC beta-lactamases have similar substrate profiles to the parental enzymes from which they appear to be derived. With one exception (13), plasmid-mediated AmpCs differ from chromosomal AmpCs in being uninducible. Both ESBLs and plasmid-mediated AmpC beta-lactamases are typically associated with broad multidrug resistance (usually a consequence of genes for other antibiotic resistance mechanisms residing on the same plasmids as the ESBL and AmpC genes). A serious challenge facing clinical laboratories is that clinically relevant ESBL-mediated resistance is not always detectable in routine susceptibility tests.

Many clinical laboratories (as well as the wider medical community) are not fully aware of the importance of ESBLs and plasmid-mediated AmpCs and how to detect them; laboratories may also lack the resources to curb the spread of these resistance mechanisms (14-16). This lack of understanding or resources is responsible for a continuing failure to respond appropriately to prevent the rapid worldwide dissemination of pathogens possessing these beta-lactamases. The consequence has been avoidable therapeutic failures (sometimes fatal) in patients who received inappropriate antibiotics (17-22) and outbreaks of multidrug-resistant, gram-negative pathogens that required expensive control efforts (23).

I describe gaps in the capabilities of clinical laboratories to accurately detect and report ESBLs and plasmid-mediated AmpC beta-lactamases; discuss some of the technical difficulties involved in designing tests to detect ESBLs in organisms other than *E. coli* and *Klebsiella* spp.; correlate laboratory problems with the recent emphasis on medical cost-cutting at a time when bacterial pathogens are increasing in complexity; and propose a way to improve laboratory performance to meet the challenge of antibiotic resistance.

Laboratory Testing for ESBLs and Plasmid-Mediated AmpC beta-Lactamases

The National Committee for Clinical Laboratory Standards (NCCLS) has issued recommendations for ESBL screening and confirmation for isolates of *E. coli* and *Klebsiella* spp., and reporting confirmed organisms (24). Compliance varies widely. Many laboratories have difficulty detecting ESBL- or AmpC-mediated resistance and may be unaware of the relevant NCCLS reporting guidelines (14). No NCCLS recommendations exist for ESBL detection and reporting for other organisms or for detecting plasmid-mediated AmpC beta-lactamases.

In the United States, many laboratories await NCCLS recommendations before attempting to detect new resistance mechanisms. Thus, many clinical laboratories attempt to detect ESBLs only in *E. coli* and *Klebsiella* spp. Some researchers suggest that this is the correct approach and that even discussion of such issues is unwarranted because it causes confusion. However, other organisms possessing these resistance mechanisms do cause infections, making this stance unacceptable. Moreover, the laboratory is an early warning system, alerting us to new resistance mechanisms in

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patients. An early warning system that allows time lags of 12 or more years before new types of resistant organisms are detected is untenable. Twelve years is not an early warning, and laboratories that operate in this manner cannot meet their responsibility.

NCCLS and the Emergence of New Pathogens

Can the current deficiencies be rectified? Two issues affect laboratories: the role of NCCLS and the speed with which new types of pathogens are emerging. NCCLS's task of creating laboratory test recommendations is difficult and often underappreciated. The committee has responsibilities in the areas of regulation, standardization, and safety. It is not NCCLS's role to be at the cutting edge of research, nor would it be appropriate for it, or other similar bodies, to be overly hasty and make decisions based on inadequate data. It can take years to gather data about a new, relatively uncommon, resistance mechanism. Time is also needed for analysis and debate. Properly done, the process cannot be rushed. The problem is that bacteria are evolving or adapting faster than this process.

Today, many bacterial pathogens are more complex than a decade or two ago. Thus, previously reliable susceptibility tests may no longer be dependable. For example, there are not only new resistance mechanisms, such as ESBLs, but also isolates that produce multiple beta-lactamases. Such organisms were not encountered often, if at all, when the current NCCLS susceptibility test criteria were prepared. For example, before the 1990s, *K. pneumoniae* isolates typically produced a single beta-lactamase, SHV-1, or occasionally two beta-lactamases (25-27). Today, *K. pneumoniae* isolates that produce three to six beta-lactamases are commonplace in some centers (28-34). Such changes necessitate new or modified tests to provide accurate and clinically relevant susceptibility reports. But instead of laboratory testing methods being upgraded during the last decade, the emphasis has been on cost-cutting and downsizing. Laboratories are under pressure to use cheaper, abbreviated tests or merely to maintain the technical status quo of a decade or more ago. In centers where the newer, more complex pathogens occur, reliance on the older tests leaves patients and institutions at risk.

A More Responsive Approach

One approach to overcoming such problems would be to ensure that each laboratory has a staff member with the time, interest, and expertise to provide leadership in antibiotic testing and resistance. This person would read relevant publications, network with other laboratories, and evaluate potentially useful tests to detect new forms of resistance in the vulnerable interim period before new NCCLS-recommended tests become available. The person with this responsibility should work closely with reference laboratories, such as those of the Centers for Disease Control and Prevention or other sites with expertise. This would help to ensure that, whenever a new resistance mechanism is suspected, it would be properly checked, and the reference laboratory could provide feedback about whether the finding was "real."

Unresolved Issues

The gaps in current laboratory knowledge and testing have generated several unresolved issues. One is whether positive, but unconfirmed, ESBL screens should be routinely reported. This is a consequence of the NCCLS two-step approach to ESBL detection. The first step is a screening for reduced susceptibility to any of the recommended screening agents (cefotaxime, ceftriaxone, ceftazidime, cefpodoxime, or aztreonam). Confirmatory testing, initiated only after a positive screening result, is based on tests with combinations of screening agents and the beta-lactamase inhibitor clavulanate. This testing indirectly detects hydrolysis of a screening agent by an ESBL by demonstrating potentiation of the activity of a screening agent in the presence of the beta-lactamase inhibitor. Confirmatory testing may require up to one extra day to detect ESBLs. If the laboratory reports a positive ESBL screening result to the physician and the isolate subsequently proves to be ESBL negative, the report could lead to unnecessary use of a carbapenem. Alternatively, if the laboratory withholds the positive screening result and the isolate is subsequently confirmed as ESBL positive, appropriate therapy may have been delayed for a day. Clearly, a reporting rule cannot cover all situations. Rather, the need to report a positive screening result should be determined on a case-by-case basis using common sense and experience as guides, taking into account the patient's status, infection control considerations, and the likelihood of a positive confirmatory test (based on prior experience with isolates from the same patient population). Using a reliable, rapid confirmatory test could minimize the time required for the second-step test and lessen this reporting dilemma. Another solution would be including ESBL confirmation testing in the routine susceptibility test.

Another issue is which NCCLS screening agent should be tested. Generally, the most reliable screening agent is the most sensitive. Cefpodoxime is the most sensitive ESBL screening agent for *K. pneumoniae* and *E. coli*, but a poor screening agent for *K. oxytoca* (35). The superior sensitivity of this agent can be accompanied by poor specificity in tests with some ESBL-negative *E. coli* isolates. This is another problem arising from the two-step approach to detecting ESBLs, which could be avoided by including a confirmatory test (ideally cefpodoxime plus clavulanate for *K. pneumoniae* and *E. coli* isolates) in the routine susceptibility test (17,36).

How best to detect ESBLs in organisms other than *Klebsiella* spp. *or E. coli* has not received much attention. The inhibitor-based confirmatory test approach is the most promising detection method (37). However, with isolates of some species, clavulanate is an unreliable agent for this test. The inhibitor-based approach is most reliable for isolates that do not coproduce an inhibitor-resistant beta-lactamase, such as AmpC. High-level expression of AmpC may prevent recognition of an ESBL. This problem is more common in tests with species or strains that produce a chromosomally encoded inducible AmpC beta-lactamase (e.g., *Enterobacter, Serratia*, *Providencia*, *Aeromonas* spp., *M. morganii*, *C. freundii*, *Hafnia alvei*, and *P. aeruginosa*). With these organisms, clavulanate may act as an inducer of high-level AmpC production and increase the resistance of the isolate to other screening drugs, producing a false-negative result in the ESBL detection test (Table 1). Tazobactam and sulbactam are much less likely to induce AmpC beta-lactamases and are therefore preferable inhibitors for ESBL detection tests with these organisms (37). Another possible solution is to include cefepime as an ESBL screening agent (38). High-level AmpC expression has minimal effect on the activity of cefepime, making this drug a more reliable detection agent for ESBLs in the presence of an AmpC beta-lactamase.

Table 1. Example of false-negative, clavulanate-based test for detecting extended-spectrum beta-lactamases with an isolate producing an inducible AmpC beta-lactamase[a]

Isolate	Test agent	MIC (µg/mL)
SHV-2-producing *Enterobacter cloacae*	Ceftazidime alone	2
	Ceftazidime + 4 µg/mL clavulanate	16

[a]Source: Thomson KS, Moland ES, Sanders CC (40).

A further concern with ESBL-producing organisms other than *Klebsiella* and *E. coli* is reporting their antibiotic susceptibilities. In Table 2, the beta-lactam MICs of an SHV-3-producing *C. freundii* isolate are within the NCCLS susceptible range of ≤8 µg/mL. If the isolate were *Klebsiella* or *E. coli*, the NCCLS reporting rule would apply, and the isolate would be reported as resistant to all penicillins, cephalosporins, and aztreonam. However, there is no ESBL reporting rule for other organisms; therefore, this organism would be reported as susceptible to cefotaxime, ceftazidime, aztreonam, and cefepime. This is inconsistent. Not only does this *C. freundii* isolate produce an ESBL, it also produces a chromosomal AmpC beta-lactamase that can hydrolyze the cephalosporins and aztreonam. It therefore seems wrong to report this organism as susceptible to these agents. Moreover, when the organism was tested at a 100-fold higher-than-standard inoculum, a dramatic inoculum effect occurred, with large increases in the MICs of these agents, analogous to the inoculum effect that occurs with ESBL-producing *Klebsiella* spp. and *E. coli* (Creighton University, unpub. data). This finding adds support for reporting all ESBL-producing isolates, not just *Klebsiella* spp. and *E. coli*, as resistant to all penicillins, cephalosporins, and aztreonam.

Detecting and reporting isolates producing plasmid-mediated AmpC beta-lactamases are more difficult issues than those associated with ESBLs. Detection is technically difficult in organisms that also produce a chromosomal AmpC, since proving that an AmpC is plasmid mediated, and not the usual chromosomal enzyme, is necessary. This determination is beyond the capabilities of most clinical laboratories. However, *Klebsiella* spp. do not possess a chromosomal AmpC. This makes them convenient indicator organisms to screen when attempting to detect plasmid-mediated AmpCs. Phenotypic tests for AmpC detection are not well defined. Screening tests could be based on decreased susceptibility to cephamycins. AmpC beta-lactamases are resistant to all marketed beta-lactamase inhibitors. Therefore, negative ESBL confirmatory tests based on these inhibitors may provide indirect evidence of AmpC production, or reduced outer membrane permeability. A positive three-dimensional test result with cefoxitin demonstrates hydrolysis of cefoxitin and differentiates between AmpC production and reduced outer membrane permeability (39). If an investigational AmpC beta-lactamase inhibitor were made available for diagnostic testing, it could be used in combination with a suitable cephem to confirm AmpC production.

Susceptibility reporting may prove controversial for isolates producing plasmid-mediated AmpC beta-lactamases. Isolates that produce these enzymes can be susceptible in vitro to cephalosporins and aztreonam (Table 3). If these agents are used therapeutically for infections with such organisms, determining if they pose a treatment failure risk for patients is a priority.

Table 2. Standard and high-inoculum microdilution MICs in tests with SHV-3-producing *Citrobacter freundii* (MICs in µg/mL)[a]

Inoculum (CFU/mL)	Cefotaxime	Ceftazidime	Aztreonam	Cefepime
5×10^5	2	1	0.5	0.5
5×10^7	256	32	32	>128

[a]Creighton University, unpub. data.

Table 3. MICs associated with plasmid-mediated AmpC production in *Klebsiella pneumoniae* (MICs in µg/mL)[a]

Enzyme	Cefotaxime	Ceftazidime	Aztreonam
FOX-1	4	8	0.5
CMY-1	128	4	32

[a]Creighton University, unpub. data.

Conclusions

Since clinical laboratories are first to encounter bacteria with new forms of antibiotic resistance, they need appropriate tools to recognize these bacteria, including trained staff with sufficient time and equipment to follow up important observations. Because bacterial pathogens are constantly changing, training must be an ongoing process. As we have learned from ESBLs, the methods and training that were previously adequate may no longer suffice against the newer types of pathogens. If laboratories continue to lag years behind new bacterial developments, new pathogens will spread, resulting in increasing problems and costs for patients and institutions.

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