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CON: Testing for ESBL production is unnecessary for ceftriaxone-resistant Enterobacterales

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Phenotypic testing for Enterobacterales that harbour ESBLs is not additive to accurate *in vitro* β-lactam MICs for clinical decision-making. ESBL testing is an outdated practice established in an era of higher cephalosporin breakpoints to prevent resistant Enterobacterales carrying Ambler class A β-lactamases with affinity for later-generation β-lactams from being reported as susceptible to later-generation cephalosporins, leading to clinical failures. ESBL testing is problematic because of inaccuracies when multiple classes of β-lactamases are produced by the same organism, thus limiting the testing application to specific species and resistance types. Clinical laboratories should instead focus finite resources on accurate susceptibility testing using contemporary interpretative criteria to help guide therapeutic decisions. With continued emergence of antimicrobial resistance and in the setting of accurate susceptibility testing and current breakpoints the use of ESBL phenotypic testing is not helpful in clinical decision-making.

There has been a long-standing debate about whether *in vitro* testing for the presence of ESBLs in Enterobacterales is additive to clinical care. We will defer the infection control issue as one for surveillance and public health and focus on the role ESBL testing in clinical practice. We argue that, for several reasons, ESBL testing is not useful for clinical care due to inaccuracies when multiple mechanisms exist, superiority of contemporary breakpoints for predicting clinical success, and variable implementation across laboratories. These challenges make ESBL testing of questionable, if any, benefit to clinical decision-making, which is the central role of the clinical microbiology laboratory. We advocate for the use of accurate updated and often lower MIC susceptibility criteria as a consistent method to identify resistance and likely clinical failure of cephalosporin or piperacillin/tazobactam therapies when directed against ESBL-producing Enterobacterales.

What is ESBL testing?

The term ESBL, particularly by clinicians, is applied in many different contexts and often means different things to different people. The most appropriate definition which is supported by testing in clinical laboratories utilizes the ESBL test for detecting Ambler class A extended-spectrum enzymes in Enterobacterales that are not carrying a chromosomal AmpC.[1] This is an important distinction from the class C cephalosporinases (AmpC) which often do not efficiently hydrolyse cefepime.[2] The traditional test involves testing *Escherichia coli*, *Klebsiella* spp. (not *aerogenes*), and *Proteus mirabilis* with cefotaxime, ceftazidime, ceftriaxone or aztreonam MIC $\geq$2 mg/L or cefpodoxime $\geq$8 mg/L ($\geq$2 mg/L for *P. mirabilis*). Isolates that screen positive using the aforementioned criteria are then evaluated by measuring the growth-inhibitory zones around both cefotaxime and ceftazidime discs with and without clavulanate. An increase in zone diameter of 5 mm or more for either agent in combination with clavulanate versus the zone diameter when tested alone indicates the presence of an ESBL.[1] The original guidance then stated to report all cephalosporins (except cephamycins) as resistant if the ESBL test was positive.

The MIC is what matters

Historically, CLSI and EUCAST suggested performing ESBL testing *in vitro* in suspected isolates describe as above.[1] The original cephalosporin breakpoints had generally higher MICs and were set prior to the widespread emergence of ESBL enzymes in Enterobacterales. The susceptibility criteria were also determined before the use of modernized pharmacokinetic and pharmacodynamics (PK/PD) to assist in setting breakpoints.[3] With the variability and inoculum effect, which occurs with susceptibility testing in the context of ESBLs, a truly resistant isolate could be categorized as susceptible especially in the context of automated

systems in use in most clinical laboratories.[4] As ESBLs continued to emerge and become more widespread, the need to re-assess clinical breakpoints followed as ESBL production led to clinical failures utilizing the older higher breakpoints.[5] By 2010, both CLSI and EUCAST determined that it was better to utilize lower breakpoints to capture many of these organisms and remove the burden of the ESBL confirmatory test from clinical laboratories.[6] This decision was multifactorial but arose, in part, from data showing many labs were not routinely performing the recommended ESBL confirmatory testing.[7] Furthermore, these new more conservative breakpoints supported by PK/PD constructs create a scenario where an organism reported as susceptible but producing an ESBL would have an MIC falling within the desired PK/PD targets, making clinical treatment success likely.[8–10] In addition, the newer breakpoints relied heavily upon PK/PD analyses that suggested that higher doses of agents than those used to set the breakpoints (1 g q12h cefepime) but commonly used clinically (2 g q12h or 2 g q8h cefepime) would provide a substantial PD cushion when these commonly used higher doses are utilized therapeutically and the lower clinical breakpoint is reported by the clinical laboratory. Lastly, the clinical failure rate of organisms with low MICs despite the presence of ESBLs is substantially lower.[4,11,12] However, even today many laboratories have not adopted the newer lower breakpoints, and may still perform ESBL testing that we would argue is both unnecessary and potentially clinically dangerous.[13,14]

When multiple mechanisms are present *in vitro* ESBL testing is inaccurate

Another of the challenges surrounding ESBL testing involves inaccuracies of the testing in organisms with multiple β-lactamase enzymes from differing Ambler classes.[15] As antimicrobial-resistant Enterobacterales continue to evolve, the occurrence of organisms where multiple mechanisms of resistance are in play becomes increasingly likely. This further decreases the value of ESBL confirmatory testing in its current iteration.[16,17] Several modifications have been proposed to improve accuracy with the presence of multiple enzymes, however it is nearly impossible to make these standard across labs with the rigor needed for clinical testing.[16,18]

The reason for choosing a narrow focus of organisms originally was the presence of many Enterobacterales with chromosomally expressed AmpC (e.g. *Enterobacter* spp.), which markedly limit the accuracy of the test and the ability to detect a class A enzyme.[17,19] ESBL testing is only valid for *E. coli*, *Klebsiella* (non-*aerogenes*) spp. and *P. mirabilis*, however other Enterobacterales are certainly capable of acquiring class A ESBLs.[20] Thus to have a test only focused on a subset of the organisms that may acquire an ESBL causes confusion among clinicians thinking that only specific Enterobacterales carry ESBL. Recent data demonstrate that *Enterobacter* spp. collections frequently harbour additional ESBLs.[21] It was also demonstrated that in *Enterobacter* spp. class A ESBLs were generally seen only at the higher MICs.[22] Therefore, we postulate that the MIC alone is more likely to be helpful in determining the presence or absence of class A ESBL enzymes in organisms with expressed chromosomal AmpC, such as *Enterobacter* spp. where a traditional ESBL test does not perform well.

Plasmid-mediated AmpC enzymes in Enterobacterales represent an additional challenge to phenotypic ESBL confirmatory testing. Since Ambler class C cephalosporinases are poorly inhibited by clavulanic acid the ESBL test in its current form will not identify these organisms.[23] Within this area one common argument in favour of ESBL testing involves the antibiotic stewardship perspective that a negative result allows for the clinical use of cefepime in third-generation cephalosporin-resistant Enterobacterales and limits carbapenem use. This argument is flawed in that it implies that a low MIC in the presence of one enzyme is fine (pAmpC) however in another (class A ESBL) the result is not to be trusted clinically. This argument is invalidated by multiple PK/PD animal studies, which suggest that irrespective of the enzyme present the PK/PD parameter associated with microbiological and clinical success remains unchanged.[8,10]

The best contemporary demonstration that ESBL testing is unhelpful in current practice is the recent failure of piperacillin/tazobactam in a trial versus meropenem for the treatment of patients with ceftriaxone-resistant *E. coli* or *Klebsiella pneumoniae* bacteraemia (MERINO).[24] This landmark trial stopped early after an interim analysis revealed that piperacillin/tazobactam did not meet its non-inferiority endpoint compared with meropenem for the treatment of bacteraemia. Subsequent analysis of trial isolates revealed inaccuracies in piperacillin/tazobactam susceptibility by automated systems compared with reference broth microdilution. This discrepancy appears to be largely driven by isolates harbouring bla_{OXA-1} in addition to ESBL enzymes.[25] As OXA-1 does not have extended-spectrum cephalosporin activity and is poorly inhibited by tazobactam, typical *in vitro* ESBL tests with a cephalosporin with and without clavulanate would not have been additive for detection of this enzyme.[4,26] It is not clear if any readily available additional *in vitro* test would reliably detect the presence of OXA-1 and warn of potential clinical failure. The trial findings demonstrate the importance of the broth microdilution MIC results and the need to improve clinical care by ensuring that MICs are accurately determined. Rather than performing additional tests that would not have predicted this failure we would argue that working toward more reliable MICs from automated systems when ESBLs are present remains essential.[27] If this is not possible then it becomes more important to have rapid genetic detection of particularly problematic enzymes rather than phenotypic tests that will be challenging to interpret across species and scenarios when multiple enzymes are present and in almost infinite combinations.

ESBL testing is not always practical

Lastly, there are several practical and logistical challenges to ESBL testing. Even when it was recommended that ESBL testing occur, less than half the clinical laboratories surveyed in the USA were doing this consistently.[7,28] It is important that clinical laboratories focus on providing consistent accurate MICs and use up-to-date susceptibility criteria, especially when there may not be the local oversight expertise to assist in the interpretation of challenging phenotypic results. Adoption of newer breakpoints will be more helpful for understanding emergence of resistance than inconsistent ESBL testing within and across laboratories.[13] In addition, if ESBL testing is done by disc diffusion it adds another day before susceptibilities can be confirmed, which delays interpretation of

susceptibilities. An *in vitro* ESBL test is present on some automated susceptibility cards but the accuracy is variable and still subject to the challenges around multiple enzymes and thus often needs to be repeated.[15,29] Laboratories should focus their finite efforts and resources on ensuring that new susceptibility interpretive criteria are quickly adopted, which will provide consistency across laboratories and improve patient safety.[13]

In conclusion, we see very little utility in performing phenotypic ESBL testing in a modern clinical microbiology laboratory. In a real world of finite resources and emerging resistance we argue that to improve clinical outcomes clinical microbiology laboratories should focus on the use of accurate methods for determining MICs and the adoption of up-to-date breakpoints for their interpretation rather than relying on phenotypic ESBL testing.

Transparency declarations

A.J.M. and J.S.L. have no conflicts to declare but are both voting member volunteers for CLSI.

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