Title: Determining the Optimal Ceftriaxone MIC to Trigger ESBL Confirmatory Testing

Running Title: ESBL Testing

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Abstract

As routine testing of clinical isolates for ESBL production (screen plus phenotypic confirmatory testing) is no longer required, a number of clinical microbiology laboratories use ceftriaxone minimum inhibitory concentrations (MICs) as proxies for identifying bacteria as potential ESBL producers. Data from our institution suggest that a ceftriaxone MIC cutoff of 8 µg/ml is an excellent predictor of ESBL production with a positive predictive value and negative predictive value approaching 100% and 99.5%, respectively.

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In June 2010, the Clinical Laboratory and Standards Institute (CLSI) lowered the ceftriaxone (and cefotaxime) breakpoint from 8 µg/ml to 1 µg/ml, and with that change, the recommendation for screening for extended-spectrum β-lactamase (ESBL) production became optional. This was in part due to the large workload imposed on clinical microbiology laboratories with phenotypic confirmatory ESBL testing. However, identification of organisms producing ESBLs still has important clinical implications. A portion of these organisms retain in vitro susceptibility to piperacillin-tazobactam and cefepime, even though these are generally considered inferior agents to carbapenem therapy for the treatment of invasive infections by ESBL-producing organisms.

Without a method of alerting clinicians to the possibility that an agent may be an ESBL producer, potentially suboptimal therapy (e.g., cefepime or piperacillin-tazobactam) may be inadvertently prescribed. In addition, the absence of institutional epidemiological data...
on ESBLs can hamper efforts to control their spread within healthcare facilities. For these reasons, many clinical microbiology laboratories use ceftriaxone (or other third-generation cephalosporin) MIC thresholds to indicate to clinicians that an organism is a possible ESBL producer or to trigger additional confirmatory phenotypic testing. If the ceftriaxone MIC threshold is lower than necessary, this practice has the potential to lead to the overuse of carbapenems or to needlessly increase the workload of microbiology laboratories (for institutions where confirmatory testing is still performed). If the ceftriaxone MIC threshold for ESBL identification is too high, appropriate infection control and treatment strategies may not be deployed. Our objective was to evaluate the sensitivity and specificity of various ceftriaxone MICs in determining the optimal threshold for identifying an organism as a potential ESBL producer.

**Microbiology methods.** Blood isolates growing *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* from January 2007-December 2013 were included. These organisms were selected as the CLSI recommended method for initial screening and phenotypic confirmatory testing is limited to these organisms. Clinical samples were processed at the Johns Hopkins Hospital Microbiology Laboratory according to standard operating procedures. Antimicrobial susceptibility testing was determined by the BD Phoenix Automated System (BD Diagnostics, Sparks, MD). *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis* organisms with MICs $\geq 2 \, \mu g/ml$ for ceftriaxone underwent further screening for ESBL production. A decrease of $>3$ doubling dilutions in the MIC for
either ceftriaxone or ceftazidime tested in combination with 4 µg/ml of clavulanic acid, versus its MIC when tested alone, was used to confirm ESBL status. The Chi-square test was used to analyze categorical variables. A two-tailed p-value of <0.05 was considered statistically significant. A Receiver operating characteristic (ROC) curve was used to determine the optimal breakpoint for the detection of ESBL producing organisms using various ceftriaxone MICs. The discriminatory power was evaluated by the area under the ROC curve (AUC) with an AUC value of 0.5 indicating no discriminative ability and an AUC exceeding 0.8 indicating good to excellent prediction. The sensitivity and specificity of the prediction rule were calculated at various ceftriaxone MIC values.

During the seven year study period, there were 1386 unique episodes of bacteremia with Escherichia coli, Klebsiella species, or Proteus mirabilis at The Johns Hopkins Hospital. After 18 cases of carbapenem-resistant Enterobacteriaceae were excluded, there were 270 and 1116 cases of ESBL and non-ESBL producing bacteremia, respectively. The proportion of ESBL producing organisms was 12.9% in 2007 and 21.9% in 2013 (p < 0.01), with a gradual increase observed annually. Evaluating all bloodstream isolates, 20.0% of E. coli, 19.1% of Klebsiella spp., and 19.1% of P. mirabilis were ESBL producers. The median MIC of ceftriaxone among ESBL producing organisms was 64µg/ml. The distribution of MICs by ESBL and non-ESBL producing organisms is depicted in Figure 1.
We determined the sensitivity and specificity of detecting ESBL producing organisms using various MICs of ceftriaxone by developing an ROC curve. The AUC of the ROC curve was 0.99 (Figure 2). An MIC cutoff of 8 µg/ml had the greatest overall sensitivity, specificity, positive predictive value, and negative predictive value at 97.8%, 100%, 100%, and 99.5%, respectively. There were 5 (1.5%) ESBL producing organisms in our cohort with ceftriaxone MICs <8 µg/ml. With a ceftriaxone MIC threshold of 2 µg/ml (as recommended by the CDC to consider an organism as a potential ESBL-producer\textsuperscript{7}), the sensitivity, specificity, positive predictive value, and negative predictive value would be 98.1%, 91.6%, 73.8%, and 99.5%, respectively.

Our data suggest that a ceftriaxone MIC cutoff of 8 µg/ml against \textit{E. coli}, \textit{Klebsiella} spp., and \textit{P. mirabilis} is an excellent predictor of ESBL production with a positive predictive value and negative predictive value approaching 100% and 99.5%, respectively. With lower thresholds, even though the sensitivity of detecting ESBLs would be relatively unchanged (i.e., the likelihood of detecting additional ESBL producers would be low), the specificity of detecting ESBLs would be compromised. A decreased specificity or "overcalling" organisms as potential ESBL producers could mean a subsequent increase in carbapenem use, leading to an additional strain on our already constrained antibiotic armamentarium\textsuperscript{8,9}.

Identification of ESBL producing organisms has important implications. Because they can spread rapidly between patients in healthcare institutions\textsuperscript{6,10}, their identification...
warrants the implementation of appropriate infection control measures. Additionally, as they may appear susceptible *in vitro* to cefepime and piperacillin-tazobactam\(^2\), even though some experts believe these agents to be inferior to carbapenems for invasive ESBL infections\(^3,4\), their recognition can impact antibiotic treatment decisions.

Certain Enterobacteriaceae may express either plasmid-mediated or chromosomally-mediated AmpC \(\beta\)-lactamases\(^1\). Using the current CLSI ceftriaxone breakpoint of 1 \(\mu\)g/ml, we previously found that 100% of 96 organisms expressing AmpC \(\beta\)-lactamases had ceftriaxone MICs \(\leq\) 1 \(\mu\)g/ml\(^1\). Ceftriaxone has been found to be problematic for the treatment of these organisms but the same has not been demonstrated with broader-spectrum \(\beta\)-lactams\(^5\). Therefore, co-existence of AmpC \(\beta\)-lactamases should be unlikely to impact the ceftriaxone MIC used to trigger ESBL testing.

As our study consists of single center data, our range of MICs for ESBL producing organisms could differ from those of other centers. However, we still believe this study is important in reminding us that clinical microbiology laboratories should periodically review their institutional data to determine the most accurate ceftriaxone MIC threshold to identify an organism as potentially ESBL producing. This is true whether laboratories use ceftriaxone MICs as a proxy for possible ESBL production or whether ceftriaxone MICs are used to trigger further phenotypic testing. If ceftriaxone MIC thresholds indicating potential ESBL production are unnecessarily low, the former could lead to the overuse of carbapenems when other \(\beta\)-lactams could suffice and the latter could lead to
We realize that our findings are preliminary and we recommend that other clinical microbiology laboratories repeat our study using their institutional data and consider expanding it to other Enterobacteriaceae. If our results are replicated, then Enterobacteriaceae with ceftriaxone MICs of 8 μg/ml or less should be considered as having a low likelihood of ESBL production, and treatment should be guided by the individual organism antibiogram (i.e., piperacillin-tazobactam and cefepime can be considered if the isolate is susceptible).

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References


Figure 1: The distribution of minimum inhibitory concentrations (MICs) by extended-spectrum β-lactamase (ESBL) producing organisms and non-ESBL producing organisms, excluding carbapenem-resistant Enterobacteriaceae.
Figure 2: Receiver-operator characteristic curves for ceftriaxone minimum inhibitory concentrations for the detection of extended-spectrum β-lactamase producing Enterobacteriaceae